EFFECTS OF ULTRAVIOLET-B RADIATION ON PLANT GROWTH, SYMBIOTIC FUNCTION AND CONCENTRATION OF METABOLITES IN LEGUMES AND AN ASSESSMENT OF F1 GENERATION FOR CARRYOVER EFFECTS

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Declaration

I hereby declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the Department of Botany, University of Cape Town. It has not been submitted for any degree or examination at any other university.

Signature:_____

Abstract

Reduction in ozone layer thickness in the stratosphere and the resultant increase in ground level of biologically active ultraviolet-B (UV-B) radiation prompted research into the effect of UV-B on growth and metabolism of terrestrial plants. In this study, eight legume species including three tropical food grain legumes [*Vigna unguiculata* (L.) Walp. (cowpea), *Glycine max* (L.) Merr (soybean), and *Phaseolus vulgaris* (L.) (common bean)], two temperate pasture legumes [*Lupinus luteus* (lupin) and *Vicia atropurpurea* (vetch)], a tree [*Virgilia oroboides* (Bergius T.M. Salter] and two shrub legumes [*Cyclopia maculata* (L.) Vent (honey bush) and *Podalyria calyptrata* Willd] indigenous to Southern Africa were exposed to UV-B radiation at above and below-ambient levels, and assessed for its effects on plant growth, symbiotic function and root concentration of metabolites.

Plant exposure to moderately and highly elevated UV-B radiation showed no effects on total dry matter, nodule number, nodule mass, nodule size, N fixed, and root concentration of flavonoid-like compounds, anthocyanins, soluble sugars and starch in *V. unguiculata*, *G. max* and *P. vulgaris*. However, N concentrations, measured as %N, were markedly reduced in roots of *G. max* and *P. vulgaris*, and in the leaves of *P. vulgaris*, which contrasted the significant increase in stems and leaves of *V. unguiculata*. With *L. luteus* and *V. atropurpurea*, elevated UV-B exposures did not alter organ growth, total dry matter, and N content per plant, in contrast to the increased ($P \leq 0.05$) flavonoid-like compounds and anthocyanin concentrations in roots and leaves of *L. luteus*, but not *V. atropurpurea*. Exposing plants to the highly elevated UV-B radiation reduced leaf and stem dry matter in C. *maculata* and %N in leaves of *V. oroboides*. The concentration of flavonoid-like compounds, soluble sugars and starch in *C. maculata*, *V. oroboides* and *P. calyptrata* were not altered with exposure to elevated UV-B radiation. Below ambient UV-B decreased total plant dry matter, nodule number, nodule dry mass, N fixed and concentration of starch in roots of *V. unguiculata* relative to both visible and UV-A radiation controls, whereas in *G. max* and *P. vulgaris*, these were not altered. Concentration of N in nodule of *C. maculata*, and in stem of *P. calyptrata* also decreased under below ambient UV-B relative to UV-A but not PAR (photosynthetically

active radiation) control. Taken together, growth and symbiotic function of all the test species except *C. maculata* were not altered with exposure to above ambient UV-B radiation.

The exposure of *V. unguiculata* and *G. max* to moderately or highly elevated UV-B radiation increased the concentration of mineral nutrients such as N, P, K, Ca, Na and B in leaves and stems, in contrast to the decreased levels of N, P, K, and B in roots. Under below ambient UV-B conditions, the concentration of K and Ca increased in stems of both *V. unguiculata* and *G. max* and in leaves of the latter with plant exposure to below ambient UV-B relative to UV-A but not PAR controls. Although elevated UV-B radiation altered nutrient concentrations in different plant organs of these species, growth and symbiotic function were not affected. Similarly, seed production and yield components of *V. unguiculata* and *G. max* were not affected except seed number in plants of the latter, which decreased with exposure to elevated UV-B. To determine whether UV-B-induced effects can be carried over to subsequent generations, plants of *V. unguiculata* and *G. max* were grown in a polycarbonate-clad greenhouse that excluded any ultraviolet radiation. Previous parental exposure to elevated UV-B radiation increased leaf flavonoids but reduced nodule and whole plant N content as well as N fixed in F1 generation of *G. max* plants, growth however remained unaltered. Additionally, shoot and whole plant $\delta^{15}N$ of these F1 generation plants also changed with previous exposure to elevated UV-B. This suggests that subtle changes in N metabolism might have occurred with potential to accumulate in progenies with further exposures to UV-B radiation.

The response of purely symbiotic and NO_3-fed nodulated plants of *L. luteus*, *V. atropurpurea*, *C. maculata*, *P. calyptrata* and *V. oroboides* to UV-B radiation varied with species. For example, NO_3-fed nodulated plants of *L. luteus* to elevated UV-B increased growth and total N content, in contrast to reduced growth and total N content in C. maculata, while *V. atropurpurea*, *P. calyptrata* and *V. oroboides* remained unaltered. From these findings, it appears that the impact of the anticipated increase in UV-B radiation on legume growth and symbiotic function in agricultural and natural eco-

systems whether from NO_3 application or atmospheric deposition is likely to be species specific.

Overall, the data of this study show little or no evidence of UV-B-induced decrease in plant growth, symbiotic function and crop yield. As a result, production of grain, pasture and tree legumes as well as the functioning of symbiotic microbial systems may not be affected with the increases in UV-B radiation.

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CHAPTER 1

GENERAL INTRODUCTION

1 General Introduction

Ultraviolet-B (UV-B; 280-315 nm) radiation is an environmental factor that has gained importance in plant research because its elevated influx to the bioshpere is shown to cause adverse effects on plant growth and development. The sun emits UV-B radiation together with UV-C (1-280 nm), UV-A (315-400 nm), visible light (400-750 nm) that is used by plants for photosynthesis, and infrared (750-25,000 nm) which is not visible to the human eye. The short wavelengths UV-C and UV-B are highly energetic and dangerous to living cells (L'Hirondelle and Binder 2002). Consequently, plants are at great risk to the harmful effects of ultraviolet radiation because of their dependence on solar radiation for photosynthesis. Fortunately, all UV-C and most of UV-B are absorbed by ozone layer in the stratosphere before they reach the earth surface (Teramura 1980). Ozone molecule, consisting of three oxygen atoms bound together, reacts strongly with other molecules. Large concentrations of ozone near the ground could be toxic to living things (National Academy of Sciences 2002; Pausch *et al.* 1996). However at higher altitudes (stratosphere), ozone is beneficial to living organisms by absorbing harmful UV radiation.

Reduction in the ozone layer thickness in the stratosphere and the resultant increase in ground level of biologically effective UV-B radiation (Madronich *et al.* 1998) has been reported in both the Southern and Northen hemispheres (McKenzie *et al.* 1999; Seckmeyer *et al.* 1994). It is established that the depletion of the ozone layer, which is the principal attenuator of solar UV-B (Teramura 1980) is due to the use of man-made air pollutants such as chlorofluorocarbons (CFCs), carbon tetrachloride and methyl bromide (McConnell and Schiff 1978; Molina and Rowland 1974) that were used in aerosol propellants, refrigeration systems, spray cans, foam packaging, and fumigation activities (Frederick 1990; Cicerone *et al.* 1974). Chlorofluorocarbons, for example, break down into chlorine atoms and residual fragments by the high energy UV radiation from the sun at altitudes above 25 km (National Academy of Sciences 2002). The chlorine atoms induce the decomposition of ozone molecules into oxygen molecules in a net chain reaction in which the chlorine atoms are regenerated and decomposition of ozone continues. As a result of that chain reaction, a single chlorine atom can destroy

as many as 100, 000 molecules of ozone (National Academy of Sciences 2002; Coohill 1991). The decline in stratospheric ozone layer was confirmed by satellite measurements (Molina and Molina 1992), with the highest reduction (71% depletion) measured over the Antarctic continent during the Southern Hemisphere spring (Kerr 1993).

Results from intensive and thorough research on ozone and the atmosphere showed a global environmental problem with a potential to catastrophic consequences and prompted countries signing an international agreement, *the Montreal Protocol* in 1987, which led to a complete ban in production of CFCs from January 1996 (National Academy of Sciences 2002). Assuming full compliance with the 1987 Montreal Protocol on Substances that Deplete the Ozone Layer and its 1992 amendments, it was predicted that ozone depletion at northern mid-latitudes will peak at the turn of the century and be 12-13% less in winter/spring and 6-7% less in summer/autumn relative to 1960 (Madronich *et al.* 1994). Stratospheric ozone might return to pre-depletion concentrations by the end of the 21st century (Madronich *et al.* 1994). However, continued implementation of the Montreal Protocol remain uncertain (Greene 1995; Jordan 1995) and the discovery of other factors such as nitrogen oxides (Tabazadeh *et al.* 2000; Waibel *et al.* 1999) and global warming (Austin *et al.* 1992) as culprits in the ozone destruction may delay the ozone recovery. The complex reactions and interactions which are not yet fully understood in atmospheric chemistry (Wennberg *et al.* 1994), including interactions among ozone and greenhouse gases (Kerr 1998) make it difficult to predict when ozone layer will fully recover. Consequently, increased levels of UV-B radiation will continue to reach the earth surface in the foreseeable future.

UV-B radiation is an environmental stress that affects plants both at the molecular and ecosystem level (Caldwell *et al.* 1998). UV-B radiation may penetrate plant tissue and directly damage cellular macromolecules including proteins and enzymes, induce DNA damage by producing pyrimidine cyclobutane dimers, increase oxygen free radical concentration and generally disrupting their biochemical and physiological mechanisms (Bornman and Sundby-Emanuelsson 1995; Sancar and Sancar 1988). In order to know

the extent of UV-B energy at damaging cells, living organisms were exposed to almost monochromatic radiation within defined wavelength band and damage response curve called action spectrum was measured (Quaite *et al.* 1992; Diffey 1991). This curve can be multiplied by the radiation energy reaching the earth surface for each wavelength, to give a weighted function called biologically effective UV-B ($UV\text{-}B_{BE}$) (Caldwell and Flint 1997) and is expressed as watts per metre squared ($W\ m^{-2}$) or kilojoules per metre squared per day ($kJ\ m^{-2}d^{-1}$) (L'Hirondelle and Binder 2002). Studies have shown that effects of elevated UV-B radiation on photosynthesis, plant growth and development vary intra- and inter-specifically between species and cultivars and are modified by other factors such as prevailing growth conditions, geographical location and interaction with other environmental factors (Caldwell *et al.* 1998; Ziska 1996). As a result, UV-B effects have been found to be detrimental to some species, beneficial or neutral to others (Musil and Wand 1994; Teramura and Sullivan 1994; Lovelock *et al.* 1992; Larson *et al.* 1990;) This has made generalization of UV-B effects difficult.

Known effects of UV-B radiation

Photosynthesis

Plants require sunlight for photosynthesis and consequently, they are exposed to harmful UV radiation that is present in the sunlight. Effects of UV-B on plant photosynthesis have been extensively researched and detailed reviews are available (Allen *et al.* 1998; Jordan 1996; Teramura and Sullivan 1994; Tevini and Teramura 1989;). Reductions in photosynthesis, measured as CO_2-assimilation, have been demonstrated in many plant species (Feng *et al.* 2002; Schumaker *et al.* 1997; Singh 1996; Cen and Bornman 1990). There is evidence that UV-B radiation can decrease the performance of all three component processes of photosynthetic machinery namely: the photophosphorylation reactions of the thylakoid membrane, the CO_2-fixation reactions of the Calvin cycle, and stomatal control of CO_2 supply (Allen *et al.* 1997; He *et al.* 1994; Middleton and Teramura 1993).

Several studies have indicated that UV-B radiation predominantly attacks the PSII reaction center and suggested PSII damage as the major potential limitation to photosynthesis (Teramura and Sullivan 1994; Melis *et al.* 1992, Stapleton 1992; Iwanzik *et al.* 1983). As a result, absorption of photosynthetically-active photon flux density (PPFD) by the antenna complexes, electron transport chain and oxidation of water are adversely affected (Allen *et al.* 1998). A decline in Rubisco activity due to decreased amount of Rubisco present and/or maximum carboxylation velocity, and a reduction in stomatal conductance in response to UV-B radiation have been demonstrated (Feng *et al.* 2002; Savitch *et al.* 2001; Allen *et al.* 1997; Schumaker *et al.* 1997; Quick *et al.* 1991). A decreased capacity for CO_2 assimilation can lead to down-regulation of organic acid, soluble sugar and sucrose biosynthesis (Savitch *et al.* 2001; Takeuchi *et al.* 1989), as well as reduced capacity for starch mobilization and impairment on the translocation of soluble sugars (He *et al.* 1994). It is therefore possible that UV-B effects on carbohydrate accumulation were indirect and arise from effects of UV-B on photosynthesis (Mackerness *et al.* 1997). However, unaltered photosynthetic productivity with supplemental UV-B radiation was also observed in some studies (Gonzalez *et al.* 1998; Nogue's *et al.* 1998; Dillenburg *et al.* 1995). This was supported by absence of UV-B effects on apparent quantum yield, photosystem II electron transport, ribulose 1,5-bisphosphate regeneration, stomatal conductance, and leaf soluble sugars and starch (Allen *et al.* 1999, 1998; Hunt and McNeil 1998; Wand *et al.* 1996).

Plant growth

Plant growth characteristics such as biomass accumulation, plant height and leaf area are altered in UV-B sensitive species. Subtle UV-B induced effects on physiological processes could accumulate and results in significant effects on biomass accumulation because total biomass represents a long-term integration of all biochemical, physiological and growth parameters (Teramura 1983). Besides alterations in the photosynthetic process, which is the source of plant carbon, UV-B induced growth responses are associated with changes in cell division and/or cell elongation (Laakso and Huttunen 1998; Tevini and Teramura 1989). This is as a result of the growth

regulator indole-3-acetic acid (IAA) which absorbs in the UV-B waveband, being converted to various photo-oxidation products including 3-methyllene-oxindole (Tevini *et al.* 1989). This photoproduct is known to inhibit hypocotyl growth when applied exogenously (Tevini and Teramura 1989). In addition, UV-B induced DNA damage and oxidative stress have also been implicated as potential causes of growth reduction (Mazza *et al.* 1999).

A number of studies have shown that total or organ biomass in different plant species and cultivars was decreased with UV-B exposure. For example, plant exposure to elevated UV-B radiation was reported to reduce biomass accumulation in some cultivars of leguminous species including *Glycine max*, *Phaseolus mungo*, *Vigna radiata*, and *Pisum sativum* (Feng *et al.* 2002; Mepsted *et al.* 1996; Singh 1996; Teramura and Murali; 1986; Biggs *et al.* 1981; Vu *et al.* 1981); cereal crops such as *Oryza satiza*, *Zea mays*, *Sorghum vulgare* (Correia *et al.* 2000; Ambasht and Agrawal 1997; Teramura *et al.* 1991); and vegetables namely *Cucumis sativus* (Murali and Teramura 1986). Even ambient levels of UV-B were found to decrease plant biomass in *V. unguiculata*, *Hordeum vulgare*, *Lycopersicum esculentum* seedlings, *Cucumis sativus* and *Lactuca sativa* (Lingakumar *et al.* 1999; Mazza *et al.* 1999; Krizek *et al.* 1998, 1997; Ballaré *et al.* 1996). Reductions in plant height and leaf area with plant exposure to UV-B radiation have also been reported in several studies (Krizek *et al.* 1997; Mepsted *et al.* 1996; Lydon *et al.* 1986;Tevin *et al.* 1986; Barnes *et al.* 1988). However, plant height or leaf area did not always correlate with biomass and therefore, decreases in height or leaf area do not always lead to reductions in total plant biomass.

Similarly, variations in tree and/or shrub response to UV-B radiation has also been reported. In a study that aimed at assessing the sensitivity of 12 tree species to elevated UV-B radiation using parameters that included plant height and biomass production, three tree species were ranked as most sensitive with only one rated as most resistant (L'Hirondelle and Binder 2002). Reductions in plant height, leaf area and/or total dry matter in tree species has been shown in conifer species that include important forest

species such as lodgepole pine (Sullivan *et al.* 1996; Naidu *et al.* 1993; Sullivan and Teramura 1989) and in *Acacia tortilis* (Ernst *et al.* 1997).

In some cases, UV-B effects on plants have been beneficial and quite opposite to those commonly expected. Al-Oudat et al. (1998) observed that plant height, leaf area and dry matter of both *Vicia faba* and *Triticum durum* increased with plant exposure to elevated UV-B radiation. Enhanced UV-B also increased needle areas of drought stressed Mediterranean pine species (Björn *et al.* 1997; Manetas *et al.* 1997). In a growth chamber study, near-UV radiation was reported to enhance the growth of P. sativum (Shiozaki *et al* 1999). However, absence of UV-B effects on plant growth parameters has also been reported in some plant species and cultivars. These reports include unaltered biomass in *P. sativum* (Allen *et al.* 1999; Stephen *et al.* 1999; Nedunchezhian and Kulandaivelu 1997; Day *et al.* 1996), *G. max* (Sullivan and Teramura 1990), *Vigna unguiculata* (Premkumar and Kulandeivelu 2001), *Oryza sativa* (Kim *et al.* 1996); *Hordeum vulgare* (Stephen *et al.* 1999), *Manihot esculenta* (Ziska *et al.* 1993) as well as in tree species of *Populus* clones (Schumaker *et al.*1997) and African *Acacia karroo* (Wand *et al.* 1996).

Reproductive processes

Ultraviolet-B radiation can alter plant reproductive processes by its effects on photosynthesis and ultimately reducing available carbon for reproduction; reducing pollen production, germination, tube length; decreasing total number of flowers produced per plant; and changing flowering phenological properties such as period to first flower and time taken to maximum flowing (Conner and Neumeier 2002; Musil *et al.* 1999; Sampson and Cane 1999; Torabinejad *et al.* 1998; Demchik and Day 1996; Musil 1995; Flint and Caldwell 1986). It is generally believed that most of the reproductive parts of a plant such as ovules and pollen are well shielded from solar UV-B radiation (Flint and Caldwell 1983). For example, anther and pollen walls, sepals, petals and walls of ovaries can absorb most of the incident radiation (Day and Demchik 1996; Flint and Caldwell 1983, Martin 1970). However, pollen may be exposed to UV-B when it is transferred to the stigma and bi-nucleate pollen types are more susceptible due to their

longer time courses for germination and penetration than tri-nucleate types (Flint and Caldwell 1984). Factors that damage pollen grain and tube may include pollen tube membrane lesions resulting from direct effects of UV-B on membrane lipids or proteins (Predieri *et al.* 1995; Kramer *et al.* 1991).

The reported change in period to first flowering and time to maximum flowering with plant exposure to UV-B (Sampson and Cane 1999; Musil *et al.* 1999; Feldheim and Conner 1996) imply a shift in timing of flowering. Consequently, insect pollinators may not be available at the time of maximum flowering leading to reduced pollination success and low seed yield (Demchik and Day 1996). In contrast, increased flowering duration, total flower number and percentage fruit set have been observed in some studies (Conner and Neumeier 2002), whereas in others, absence of UV-B effect on reproductive parts were reported (Torabinejad *et al.* 1998; Feldheim and Conner 1996).

Crop yield

There are a limited number of UV-B experiments involving species covering the whole growing season and producing crop yield data. These reports show inconsistent UV-B effects on crop yields with some studies demonstrating negative, neutral or even positive effects (Conner and Zangori 1998; van de Staaij *et al.* 1997; Musil 1995; Teramura *et al.* 1990). For example, seed production increased in three of four monocotyledonous species studied, but decreased in three of four dicotyledonous species (Musil 1995). In another study involving *G. max* cv. Essex, a combined six year yield reduction of approximately 20% at a simulated 25% ozone depletion was recorded, while in cv. Williams, grain yield increased by 7% at the same UV-B dosage and by 15% at the lower UV-B dose (Teramura *et al.* 1990). These inter- or intra-species variations in UV-B effects on crop yields have also been reported in several studies with reductions in yields of some cultivars of *G. max* (Singh 1994; Teramura and Murali 1986) *Pisum sativum* (Mepsted *et al.* 1996); *Zea mays* (Correia *et al.* 2000; Mark *et al.* 1996), *Hordeum vulgare* (Mazza *et al.* 1999). In contrast, no UV-B effect on seed yield was obtained in some studies also involving *G. max*, (Sinclair *et al.* 1990), *Pisum sativum*

(Stephen *et al.* 1999), *Vicia faba* (Al-Oudat *et al.* 1998), *Oryza sativa* (Olszyk *et al.* 1996), *Phacelia campanularia* and *P. purshi* (Conner and Neumeier 2002).

Carry-over effects

There is evidence that small detrimental effects induced by elevated UV-B radiation can accumulate into substantially larger effects as subsequent generations are exposed to UV-B radiation. Sullivan and Teramura (1992) indicated that in loblolly pine, reduction in plant biomass observed after three successive years of UV-B radiation simulating a 25% ozone depletion was approximately double that observed after one year of UV-B exposure. In another study, adverse effects of UV-B radiation on biomass production and reproduction processes, and altered biomass allocation among plant organs accumulated over three generations of *Dimorphotheca sinuata* exposure to elevated UV-B radiation (Musil 1996). Because the action spectrum for inhibition of hypocotyl elongation by UV in *Lepidium sativum* resembled that obtained for DNA damage (Steinmetz and Wellmann 1986), the accumulation of UV-B effects over multiple generations was attributed to altered molecular signals resulting from DNA damage (Musil 1996). This was further demonstrated in a follow-up study where observed changes in the physiology, development and fecundity of *D. sinuata* offspring after successive exposure to elevated UV-B showed unlikely UV-B effect of photomorphogenic origin, but conceivable UV-B-induced genetic change or mutation that was carried over from one generation to another (Musil *et al.* 1999). In contrasts, beneficial effects of parental exposure to elevated UV-B radiation was reported by Conner and Neumeier (2002) where the resulting offspring of enhanced UV-B plants grew taller and produced more leaves and seed yield than the offspring of ambient UV-B plants. Since most mutation effects are deleterious, the beneficial UV-B effects on the offspring was attributed to maternal effects where UV-B effects, though not shown in the maternal plants, were invested more in their subsequent generation (Conner and Neumeier 2002) or UV-B effects on the seeds (Musil *et al* 1998).

Pigment accumulation and protection against UV-B damage

To cope with UV radiation damage, plants have developed a variety of mechanisms including screening out UV radiation by accumulating UV-absorbing phenolic compounds in leaf epidermis, repairing UV-induced DNA damage, formation of antioxidants to scavenge peroxides and oxygen radicals, increasing the path-length for the radiation by thickening the epidermis, and reflecting UV radiation by producing wax coating at the leaf surface (Olsson *et al.* 1999; Jordan 1996; Bornman and Teramura 1993; Day 1993; Haupt and Scheuerlein 1990; Mulroy 1979). One obvious target for UV-B induced damage is DNA where photoproducts such as pyrimidine cyclobutane dimers are formed (Sancar and Sancar 1988). The formation of DNA dimers may lead to changes in the base-pairing properties between the two DNA strands at the site of the lesion (Jiang and Taylor 1993). However, cellular repair mechanisms remove most of these DNA modifications by light-requiring repair enzyme photolyase through excision or recombinational repair (Sancar and Sancar 1988). Photo-repair mechanisms are activated by high PAR levels and UV-A and were reported to reduce UV-B induced damage (Mirecki and Teramura 1984, Jordan *et al.* 1992; Mackerness *et al.* 1996). Antioxidants such as glutathione, ascorbate, α-tocopherol, and carotenoids, which are reported to increase with UV-B exposure, can detoxify highly reactive oxygen species that are formed when UV-B interacts with molecular oxygen (Götz *et al.* 1999; Wildi and Lütz 1996; Strid *et al.* 1994).

The role of flavonoids in UV-B radiation protection was hypothesized several decades ago (Jagger 1967) but definite proof has only been obtained when studies involving mutants showed hypersensitivity to UV radiation. In one such study, an *Arabidopsis* mutant tolerant to lethal UV-B levels show constitutively high levels of flavonoids and other phenolics (sinapate) (Bieza and Lois 2001). The increase in flavonoids was associated with enhanced expression of chalcone synthase (CHS) mRNA under UV-B radiation suggesting that the increases in absorption may be a consequence of changes in gene expression (Bieza and Lois 2001; Strid *et al.* 1994). In another report, CHS mutants of *A. thaliana* were found to be hypersensitive towards UV-B radiation (Li *et al.* 1993), as CHS is the key enzyme that commits the pathway to flavonoid synthesis

(Beggs and Wellmann 1994). The name flavonoid is used for all compounds derived from the flavan or isoflavan skeleton (Beggs *et al.* 1986). However, in the literature on photocontrol of these pigments, the term flavonoid is often used to mean all flavonoids except anthocyanins.

Flavonoids including flavones, flavonols and isoflavonoids absorb light between 230-380 nm (Taylor *et al.* 1997) and this includes the UV-B region of solar radiation. Consequently, their accumulation in the epidermis of leaves reduce transmittance of UV-B radiation and thus protect the inner cells (Tevin *et al.* 1991). Ultraviolet-B induction of flavonoids and other phenylpropanoid derivatives such as sinapate esters in plant tissue especially leaves, is the most widespread response of plant species to UV-B radiation (; Kolb *et al.* 2001; Tegelberg and JulKunen-Tiitto 2001; Mazza *et al.* 2000; Strid *et al.* 1994; Ziska *et al.* 1992). Furthermore, anthocyanin has also been reported to increase with plant exposure to UV-B in several plant species including cotton (Bennett 1981), *Sorghum bicolor* (Drumm-Herrel and Mohr 1981) and *Zea mays*, *Triticum sativum* and rye (Wellmann 1982). Apparently, anthocyanins may not be effective protector of UV-B radiation because they have little absorption in the UV-B waveband (Teramura 1983).

Although the concentration of flavonoids or phenolics increases with UV-B exposure in most species, variation in responses of specific flavonoid were observed in some studies implying that flavonoids have different antioxidant properties (Tegelberg and Julkunen-Tiitto 2001). For instance, the ratios of luteolin:apigenin and quercetin:kaempferol derivatives, luteolins and quercetins being more efficient antioxidants, have been reported to increase under enhanced UV-B (Tegelberg and Julkunen-Tiitto 2001; Markham *et al.* 1998; Ryan *et al.* 1998). Olsson *et al.* (1998) observed that amount of quercetine glycosides was markedly higher that kaempferol glycosides in leaves of *B. napus* exposed to elevated UV-B radiation. Kolb *et al.* (2001) showed that production of hydroxycinnamic acids in leaves of *Vitis vinifera* (grapes) was stimulated by strong visible light, but biosynthesis of kaempferol and quercetin was specifically increased by UV-B radiation. Besides the protective role in defence against UV-B radiation, flavonoids play a variety of other important roles such as plant signaling

molecules to symbiotic bacteria in the Rhizobiacea (Cullimore and Dénarié 2003; Phillips 2000; Dakora and Phillips 1996) and mycorrhizal fungi (Harrison and Dixon (1994), phytoalexins (Landry *et al.* 1995; Dixon *et al.* 1983), anti-herbivore (Lavola *et al.* 1997) as well as nutraceutical compounds (Shetty *et al.* 2002). Consequently, induction of either total or specific flavonoid biosynthesis by UV-B has a potential of altering several other physiological and biochemical functions of flavonoids in plants.

UV-B and plant nutrition

Plant uptake and translocation of mineral nutrients within the plant can be affected by elevated UV-B radiation (Premkumar and Kulandaivelu 2001), but the mineral status of plants can also affect plant responsiveness to UV-B radiation (Musil and Wand 1994; Murali and Teramura 1985). Increases in plant growth under low but not high nutrient conditions have been observed in some species exposed to elevated UV-B radiation (Musil *et al.* 1999; Lavola *et al.* 1997; Musil and Wand 1994) suggesting that plant sensitivity to UV-B radiation is reduced under low nutrient conditions. Indeed sensitivity of *Cucumis sativus* and *Zea mays* to UV-B radiation increased with increased levels of N supply (Correia *et al.* 2000; Hunt and McNeil 1998;) and of *G. max* with phosphate fertilization (Murali and Teramura 1985). The lower sensitivity of N-starved plants to UV-B radiation has been attributed to lower rate of cell division (Rufty *et al.* 1989) that increase the opportunity for DNA dimers to be repaired before the cell enters the DNA synthesis (Sancar and Sancar 1988). Additionally, deficiencies in N, P, S and K have been reported to increase the production of secondary metabolites including flavonoids (Fajer *et al.* 1992; Murali and Teramura 1985) by regulating either the expression of enzymes involved in the biosynthesis of phenolic compounds or availability of substrates (Lavola *et al.* 1997). The increase in flavonoid concentration under low nutrient condition could therefore confer greater protection against UV-B damage.

UV-B and $^{15}N/^{14}N$ fractionation

It is currently known that plant $\delta^{15}N$ can be used as a physiological integrator of N metabolism in plants because $\delta^{15}N$ composition in a plant is a function of $\delta^{15}N$ values of external sources and $^{15}N/^{14}N$ fractionation that may occur during N uptake or fixation,

transportation, assimilation and loss of N from the plant (Robinson *et al.* 2000). The $^{15}N/^{14}N$ fractionation in plant tissue may be influenced by among other factors, plant physiological characteristics such as nodulation (Kumarasinghe *et al.* 1992; Yoneyama 1995), mycorrhizal infection (Evans 2001; Högberg 1990), and environmental stress factors including temperature (O'Leary 1995), rainfall (Handley *et al.* 1999), drought, and N deficiency (Robinson *et al.* 2000). The effect of UV-B on $\delta^{15}N$ composition in plants has not yet been reported. However, in C isotope studies, UV-B radiation is reported to induce more negative $\delta^{13}C$ values which was attributed to decreased water use efficiency but increased internal CO_2 (C_i) concentration (Feng *et al.* 2002; Ormrod *et al.* 1997; Naidu *et al.* 1993). Infact, $\delta^{13}C$ theories are available which can be used to interpret $\delta^{13}C$ variations among C_3 plants in-terms of measurable physical and physiological processes (Farquhar *et al.* 1982). For example, $\delta^{13}C$ has been used to screen C_3 genotypes for potential water use efficiency (Ehleringer *et al.* 1993). In contrast, the use of $\delta^{15}N$ in eco-physiological studies is still at an early stage of exploration, and documenting taxonomic and environmental variations in $\delta^{15}N$ under natural and controlled conditions (Handley *et al.* 1998) that may later be used to develop hypotheses and theories.

UV-B and plant-soil microbial symbiotic systems

Moorhead and Callaghan (1994) indicated that UV-B radiation normally did not penetrate the soil beyond 5mm of the soil surface, and therefore the effects of UV-B on soil microbial populations would be indirect. Because UV-B radiation can adversely affect photosynthesis (Feng *et al.* 2002, Schumaker *et al.* 1997; Teramura and Sullivan 1994), alter translocation and allocation of carbon to different plant organs (Adamse and Britz 1992) leading to a higher shoot/root ratio in sensitive species (Tosserams *et al.* 1996), it is likely that availability of carbon in the root for microbial use and/or amount or quality of rhizosphere exudates may change. Consequently, microbial populations associated with the rhizosphere such as rhizobia and mycorrhizal fungi would be affected since they are fed primarily by root-derived substrates (Klironomos and Allen 1995). Additionally, increased concentration of flavonoids in plant tissue as a defence mechanism against UV-B radiation may influence nodulation and N_2 fixation because

flavonoids are also associated with rhizobial *nod*-gene induction (Phillips 2000; Dakora and Phillips 1996). In fact, an increase in flavonoids in roots of *Phaseolus vulgaris*, *P. sativum* plants exposed to ambient UV-B radiation under chamber and glasshouse conditions was followed by a corresponding increase in nodule number, nodule dry matter and N_2-fixation (Pinto *et al.* 2002; Shiokazi *et al.* 1999).

It has been demonstrated that a reduction in photosynthate translocation in *G. max* inhibited N_2 fixation probably due to reduced available energy to sustain high energy requiring process such as N_2 fixation (Pausch *et al.* 1996; Streeter 1993; Mulchi *et al.* 1992). However, in a study involving mycorrhizal fungi, Klironomos and Allen (1995) did not find a significant change in total AM fungal infection of *Acer saccharum* (Sugar Maple) roots with UV-B exposure, but the morphology of the mycorrhizal fungi changed from the production of arbuscules to that of vesicles and hyphal coils. The vesicles are resting structures of mycorrhizal fungi (Brundrett 1991) and hyphal coils are longer-lived structures (Cooke *et al.* 1992), less metabolically expensive and have shown to predominate under other unrelated stressful conditions (Duckmanton and Widden 1994). This implies that the shift in the mycorrhizal-fungi morphology was a result of UV-B-induced stress on the *Acer saccharum* (Klironomos and Allen 1995).

These reports show that UV-B effect on photosynthesis, plant growth and development can be both positive and negative, and vary intra- and inter-specifically between species and cultivars. Additionally, numerous studies have demonstrated that accumulation of flavonoids and anthocyanins by plants provide a defence mechanism against UV-B radiation (Bieza and Lois 2001; Rozema *et al.* 1997). However, these molecules also serve as plant signals to symbiotic bacteria in the Rhizobiaceae (Cullimore and Dénarié 2003; Phillips 2000; Dakora and Phillips 1996), and their accumulation in root tissues has been shown to promote nodule formation (Pinto *et al.* 2002; Muofhe and Dakora 1999; Shiokazi *et al.* 1999). Recently, it has been reported that UV-B induced flavonoid-like compounds occurred not only in the leaves but also in the roots of *P. vulgaris*, and plants with leaves exposed to UV-B also significantly increased release of these compounds from the roots to the medium (Pinto et al. 2002). Thus, an increase in

concentration of flavonoids and/or anthocyanins concentration in roots of plants exposed to UV-B radiation could potentially stimulate nodulation and N fixation in legumes (Figure 1.1). But the exposure of plants to elevated UV-B has also been shown to reduce photosynthesis (Teramura and Sullivan 1994) and to alter the allocation of biomass to different plant organs (Adamse and Britz 1992) of sensitive species. The net result was a higher shoot/root ratio in plants exposed to UV-B relative to those receiving no UV-B radiation (Tosserams *et al.* 1996). An increased allocation of biomass to shoot would imply low carbohydrate supply to roots and nodules, with a consequent decrease in the release of root exudates compounds into the rhizosphere. This in turn could affect nodulation and N_2 fixation in the test species (Figure 1.1).

Even though some studies on plant responses to elevated UV-B radiation have involved legumes such as cowpea (Lingakumar *et al.* 1999), alfalfa (Quaite *et al.* 1992) and pea (Strid and Porra 1992), common bean (Cen and Bornman 1990), soybean (Teramura and Murali 1986) no attention was paid to their symbiotic function. There is, so far, only three studies on the effects of UV-B on N_2 fixation in symbiotic legumes. One study claimed that elevated UV-B radiation decreased nitrogenase activity by 78% and reduced the number and size of root nodules of *Vigna radiata* and *Phaseolus mungo* (Singh 1997). The other studies reported increased growth, nodulation and N_2-fixation in common bean (Pinto *et al.* 2002) and pea (Shiokazi *et al.* 1999) with plant exposure to ambient UV-B radiation. A report by Dakora and Keya (1997) has shown a significant contribution by N_2 fixation to soil fertility in tropical Africa. Thus, at the ecosystem level, a decrease in N_2 fixation by UV-B as suggested by Singh (1997) could result in decreased soil-N fertility from reduced nodule function. Clearly, more studies are needed to establish the direct effects of UV-B on legume nodulation and N_2 fixation.

Hypotheses

It is hypothesized that a rise in UV-B radiation could 1) increase nodulation and N_2 fixation in legumes if the increase in flavonoid concentration in roots of parent plants and in seeds of the F1 generation includes nod-gene inducers, or 2) reduce nodulation and N_2 fixation if the damaged photosynthetic machinery of legumes results in reduced

photosynthetic C supply to nodules and/or decreased release of biologically functional root exudates compounds into the rhizosphere.

Objectives

1. To determine the effects of UV-B radiation on growth, symbiotic function and concentration of metabolites in tropical grain legumes.
2. To assess the effects of UV-B radiation on concentration of macro- and micro-nutrients in different organs of *V. unguiculata* and *G. max*.
3. To determine the effects of UV-B radiation on growth, symbiotic function and concentration of metabolites of *Lupinus luteus* and *Vicia atropurpurea* plants either depending entirely on symbiotic N_2 fixation for their nutrition or fed 2 mM NO_3^-.
4. To determine the effects of UV-B radiation on growth, symbiotic function and concentration of metabolites of purely symbiotic and NO_3-fed nodulated tree and shrub legumes indigenous to Southern Africa.
5. To examine the effects of UV-B radiation on seed yield of *V. unguiculata* and *G. max* and assess carryover effects on the F1 generation progenies
6. To assess UV-B effects on $^{15}N/^{14}N$ fractionation in legume plant organs and to determine whether $\delta^{15}N$ correlates with total or individual organ dry matter, %N and N content

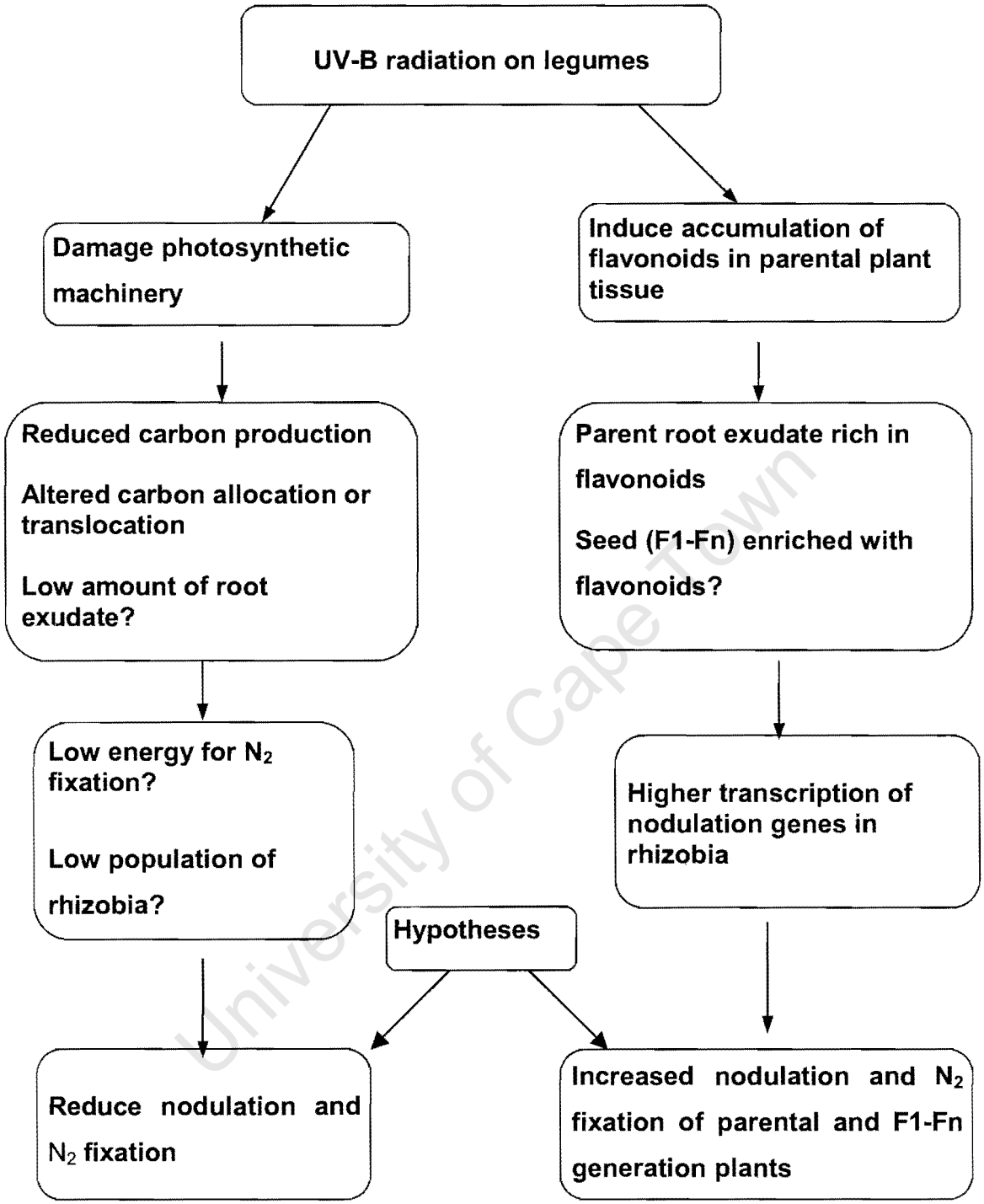

Figure 1.1. A flow diagram of possible effects of UV-B radiation on nodulation and N_2 fixation in legume plants.
? = no experimental evidence.

CHAPTER 2
MATERIALS AND METHODS

2.1 Plant species and culture

Eight legume species studied included three tropical food grain legumes: *Vigna unguiculata* (L.) Walp (cowpea) landrace (Bengpilaa ex-Ghana), *Glycine max* (L.) Merr (soybean) cv Prima and *Phaseolus vulgaris* (L.) (common bean) cv PAN 159; two temperate pasture legumes: *Lupinus luteus* (L.) (lupin) and *Vicia atropurpurea* (Desf.) (vetch); and a tree and two shrub legumes indigenous to Southern Africa: *Virgilia oroboides* (Bergius T.M.) Salter, *Cyclopia maculata* (L.) Vent (honey bush) and *Podalyria calyptrata* Willd. Seeds were sown into 20 cm high x 20 cm diameter pots containing sand and germinated under different UV-B treatments. Seeds of *V. unguiculata* were inoculated with *Bradyrhizobium* strain CB756, *G. max* with *B. japonicum* strain CB 1809 and *P. vilgaris* with *Rhizobium leguminosarum* bv. Phaseoli strain UD2, *L. luteus* with *Bradyrhizobium* lupin and *V. atropurpurea* with *Rhizobium leguminosarum* bv vicea at planting. *Cyclopia maculata*, *P. calyptrata* and *V. oroboides* were inoculated with rhizobia isolates from nodules of the same species at emergence.

Seedlings of *V. unguiculata*, *G. max*, *P. vulgaris*, *L. luteus* and *V. atropurpurea* were later thinned to one per pot, but those of *C. genistoides*, *P. calyptrata* and *V. oroboides* were thinned to two. Pots were irrigated with equal volumes of water. Immediately at seedling emergence, 400 mL of ½ strength N-free Hoagland's nutrient solution (Hewitt 1966) was supplied twice weekly to all species except *C. genistoides*, *P. calyptrata* and *V. oroboides* which received ¼ strength of the nutrient solution. Seedlings of *C. genistoides*, *P. calyptrata* and *V. oroboides* were provided with ¼ strength Hoagland's solution because these species have relatively low growth rate and are indigenously grown on low fertile areas. To avoid accumulation of nutrients in the growth media, the sand was flushed once every week with water.

2.2 Experimental UV-B treatments

There were five experiments conducted. In four of the five experiments, plants were exposed to above ambient or below ambient UV-B radiation. The fifth experiment

was carried out in a polycarbonate-clad greenhouse, which attenuates solar radiation below 400 nm to cut off all ultraviolet radiation in-order to determine whether elevated UV-B radiation effects are carried over to F1 generation.

2.2.1 *Above ambient UV-B treatments*

The experiments compared two elevated levels of UV-B (moderately and highly elevated UV-B) with a control (ambient) treatment. In the ambient control ($UV\text{-}B_{100}$), lamps in alternating banks were filtered (no transmission below 316 nm) with 0.12-mm thick Mylar-D film (DuPont De Nemours, Wilmington, Delaware, USA). In the two above-ambient treatments, lamps in intervening banks were filtered (transmission down to 290 nm) with 0.075 mm-thick cellulose acetate film (Courtaulds Chemicals, Derby, UK). Hence, plants receiving UV-B control were exposed to only ambient UV-B radiation whereas those under elevated UV-B treatments received extra UV-B from the lamps. All filters were replaced weekly to ensure uniformity of UV transmission. Artificial UV-B radiation was supplied daily for an 8-h period. Irradiation was graduated with two-thirds of the total daily UV-B supplement spread over a 4-h photoperiod centred on the solar noon. The remaining one-third was applied equally over the two 2-h early morning and late afternoon photoperiods. This was achieved by switching on fewer lamps in each bank during these photoperiods. This step-wise application of the supplemental UV-B was followed in order to simulate alterations in ambient UV-B irradiance intensity due to diurnal changes in solar zenith angle.

Spectral irradiances of filtered lamps were measured after sunset with a computer-interfaced monochromator spectroradiometer (IL-1700/IL760D/IL783 Series Spectroradiometer Systems which comprise, an IL1700 Research Radiometer, detector, monochromator, input optics, order sorting filters, and NBS traceable calibration; International Light Inc., Newburyport, USA), calibrated for absolute response and checked for wavelength alignment. Measured irradiances were weighted with the generalized plant response action spectrum (Caldwell 1971), as mathematically formulated by Green *et al.* (1974), which was normalized at 300 nm. Weighted irradiances were integrated over wavelength and expressed as a function of distance

from the lamp source. Distances between cellulose-acetate filtered lamps and median height of plants in each bank were adjusted to increase UV-B above modelled clear-sky background flux (range: 0.898 - 8.554 kJ m^{-2} d^{-1}) by 34% (UV-B_{134}: 1.270 - 11.276 kJ m^{-2} d^{-1}) and 66% (UV-B_{166}: 1.626 - 13.815 kJ m^{-2} d^{-1}). These UVB increases simulated 15% and 25% depletions respectively in total column ozone above Cape Town according to a computerized (Musil and Bhagwandin 1992) semi-empirical model (Green 1983). To avoid semi-empirical model overestimation of the level of supplementary UV-B irradiance required for each UV-B treatment due to local variations in the amount and form of cloud and atmospheric aerosols (Theil *et al.* 1997), artificial UV-B supplements were applied under predominantly clear-sky conditions. This was achieved by switching off lamps during the passage of intermittent cold fronts. The experimentally simulated depletion of stratospheric ozone exceeded the predicted 11% for all seasons at southern-hemisphere mid-latitudes (Madronich *et al.* 1995). Nevertheless, the total daily UV-B exposures supplied in the UV-B_{162} treatment did not exceed mid-summer ambient levels in the tropics. Lamps in the mylar-filtered controls (UV-B_{100}) were fixed at the same distances above plants as in the UV-B_{132} treatment to provide similar UV-A exposures in both (Newsham *et al.* 1996).

Lamp heights were regularly adjusted to accommodate increases in median height of plants in each bank and seasonal variations in UV-B exposure. Adjustments were checked with a UV-B biometer sensor (Model 3D-600, Solar Light Company, Philadelphia, USA) calibrated against the spectroradiometer for the generalized plant action spectrum, which regularly checked percentage changes in UV-B beneath the lamps. Measured UV-B exposures over the growing period of plants averaged 95.0% (range: 86.7% - 104.8%) of background in the UV-B_{100} control, 137.1% (range 127% - 149.2%) of background in the UV-B_{134} treatment and 169.3% (range: 154.0 - 186.0%) of background in the UV-B_{166} treatment (Figures 2.1A and B).

2.2.2 *Below-ambient UV-B treatments*

Chambers (1.5 m^2 square x 0.75 m high) were constructed of differentially UV-transmitting clear perspex (300 mm thick). This experiment allowed measurement of

reduced UV-B (22% of ambient) with two controls, one for photosynthetically active radiation (PAR) and the other for UV-A radiation. Since the UV-B treatment contained both PAR and UVA wavelength, both of which are known to have moderating effects on UV-B induced damage (Caldwell, *et al.* 1994; Middleton and Teramura 1994), the two controls assisted in separating the effects of these wavelengths from that of UV-B. For instance, by comparing UV-B_{22} treatment and PAR control, the effect of below-ambient ultraviolet (both UV-B and UV-A) radiation (Figure 2.2A) could be assessed, and comparison of UV-B_{22} and UV-A control permitted determination of the sole effect of only below ambient UV-B radiation (Figure 2.2A). For the below-ambient UV-B_{22} treatment, chambers were clad with an extruded grade of perspex (transmission down to 250 nm). For the PAR radiation control (PAR_{cont}), chambers were covered with a cast grade of perspex (transmission down to 372 nm), and for the UV-A radiation control (UV-$A_{cont.}$), they were covered with the extruded grade of perspex coated with a 0.12-mm thick Mylar-D film (Figure 3A). Measured changes in UV-B, UV-A and PAR radiation in the chambers against background levels as determined with the UV-B biometer sensor, PAR (LI 189, Li-Cor, Lincoln, NE, USA) are presented in Figure 2.2B. The small amount of UV-B radiation measured under UV-A and PAR controls (Figure 2.2B) was due to diffused scattered UV-B radiation coming from the open southern side of the chambers.

Average maximum daily air temperatures in the chambers (29.2°C) and background (27.7°C) were recorded using temperature sensors, and their changes in the chambers against background for each radiation treatment are presented also in Figure 2.2B. The conditions in the chambers were somewhat different from those on the tables, in that the average temperature was 1.5 degrees warmer in the chamber. The tables and chambers were inter-dispersed in an open area (Figure 2.3) at the Kirstenbosch National Botanical Gardens, Cape Town (36°56' S, 18°29' E). However, all comparisons were made within tables or chambers so that these conditions were never confounded with treatments.

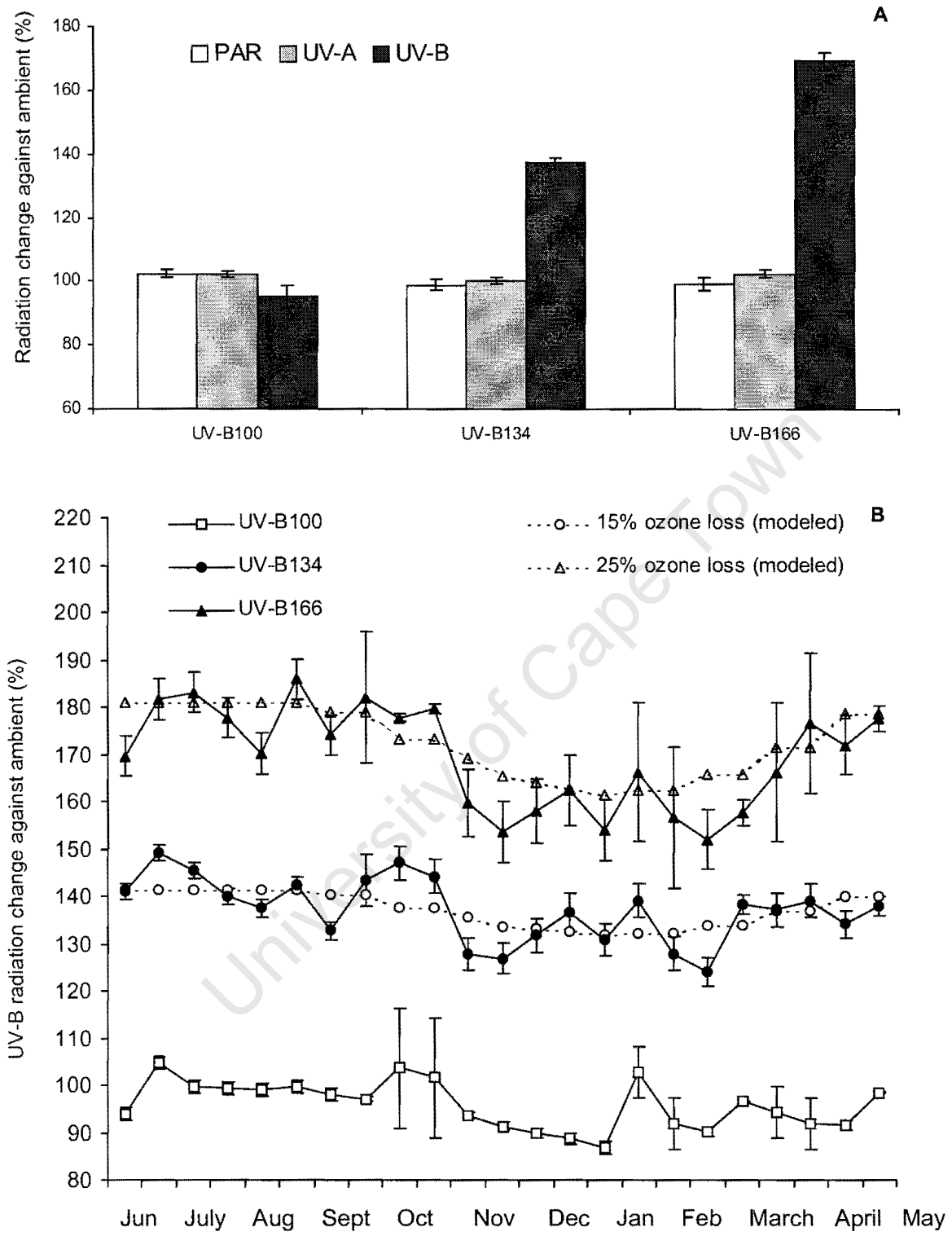

Figure 2.1. Measured changes in the UV-B, UV-A and PAR beneath lamp systems (A) and measured and modeled UV-B radiation change at 15 and 25% ozone loss (B) for the entire year.

2.3 Plant harvest and measurement of biomass

At harvested, plants were separated into nodules, roots, stems, leaves and pods. Nodules were counted, and together with the other plant organs oven-dried at 60°C to a constant dry weight. After recording the dry weights, each sample was ground into a fine powder for analysis of N and metabolites. Average nodule size was calculated as total nodule mass divided by nodule number.

2.4 Analysis of N in tissues

Concentrations of N in all plant organs and seeds were measured as %N using a carlo Erba NA 1500 elemental analyser (Fisons Instruments SpA, Strada Rivoltana, Italy) coupled to a Finniggan MAT 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) via a Conflo II open-split device. Amount of N per organ was estimated from the product of %N and the dry mass. Nitrogen fixed was calculated as total plant nitrogen minus seed nitrogen, while the rate of specific nodule N_2-fixing activity (mg N fixed $.g^{-1}$ nodule mass d^{-1}) was obtained by dividing N fixed by nodule dry matter and length of plant growth period.

2.5 Measurement of phenylpropanoids

Methanolic extract compounds (possibly flavonoids and anthocyanins) were extracted from oven-dried, ground tissue samples of plant, suspended in 10-mL volumes of acidified methanol (79:20:1, v:v, methanol:water:HCl), centrifuged and absorbances measured at 300 nm for flavonoid-like compounds (Mirecki and Teramura 1984), and 530 and 657 nm for anthocyanis (Lindoo and Caldwell 1978). Concentration of flavonoid-like compounds were expressed as absorbance (A_b) at 300 nm $.g^{-1}$ dry matter, and anthocyanins calculated as A_b530 nm – 1/3 A_b657 nm $.g^{-1}$ dry matter (Lindoo and Caldwell 1978).

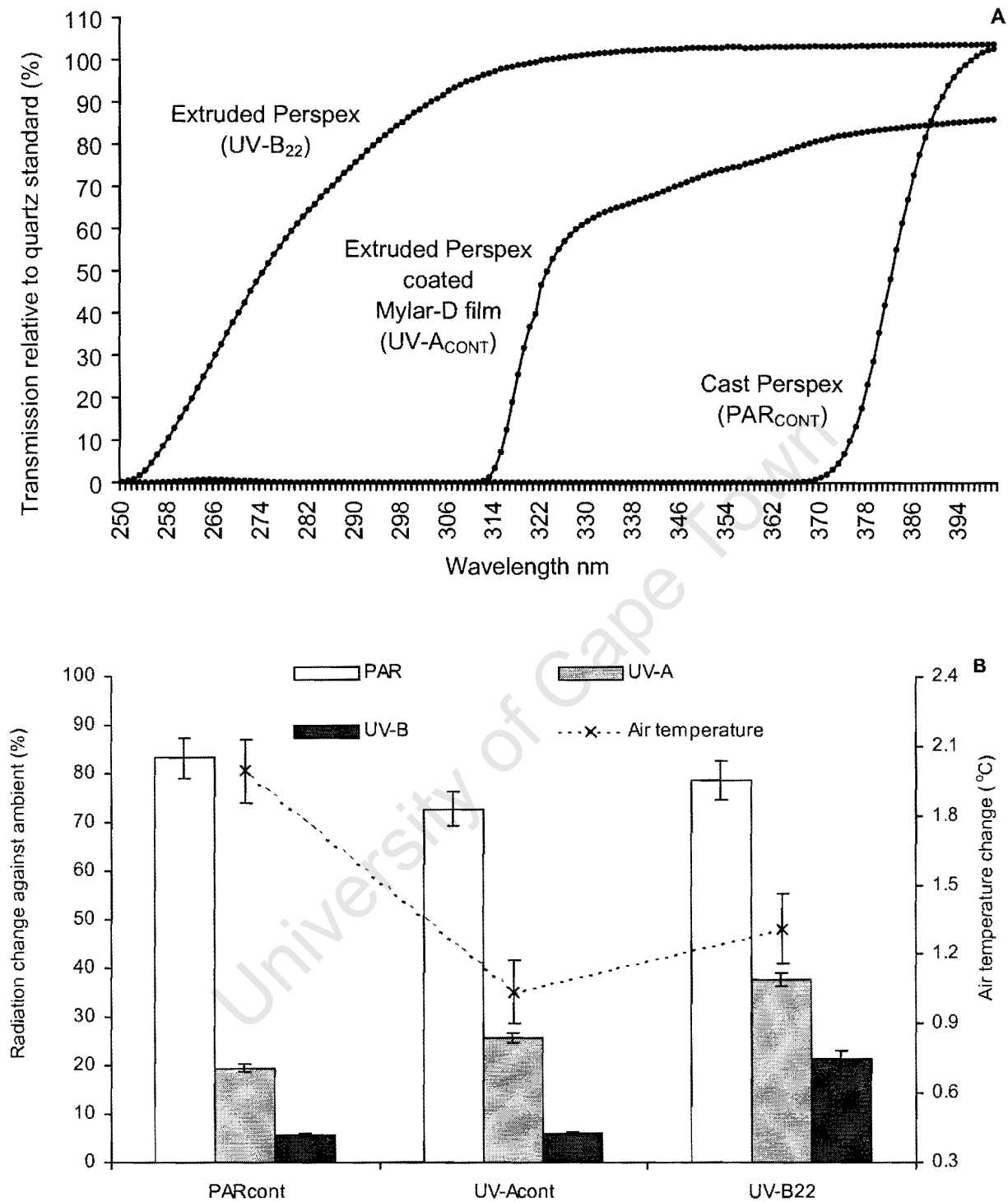

Figure 2.2. Spectral characteristics of extruded and cast grades of Perspex and mylar film used in chamber constraction (A), and measured changes in UV-B, UVA, PAR and maximum daily air temperatures in chambers (B).

Figure 2.3. Tables and Chambers interdispersed in an open area at Kirstenbosch National Botanical Gardens, Cape Town.

2.6 Measurement of non-structural carbohydrates

Total soluble sugars (sucrose, glucose and fructose) were extracted from oven-dried, ground samples of plant roots. A weighed amount was put in aqueous ethanol (0.1 g : 10 mL 80% aqueous ethanol, v/v) and kept at 0°C for at least 72 h for self-extraction. The extracts were centrifuged and the supernatant adjusted to 25 ml for spectrophotometric determination of total soluble sugars as described by Buysse & Merckx (1993). The tissue pellets from centrifugation were oven-dried at 60°C for 48 h and weighed. Starch was measured by hydrolyzing the dried pellet for 3 h in 5 mL 3.6% HCl at 100°C, centrifuging the extracts, and adjusting the volume to 25 mL for spectrophotometric determination of the resultant sugars in the extract (Buysse and Merckx 1993). Soluble sugar and starch concentrations were expressed as mg $.g^{-1}$ dry matter.

2.7. Measurement of macro- and micro-nutrients

Dry ashing followed by acid digestion was used in the preparation of plant samples for determination of macronutrients (P, K, Ca, Mg and Na) and micronutrients (Fe, Cu, Zn, Mn and B) by spectrophotometry (Giron 1973). A weighed amount (1.00 g dry matter) of plant material was placed in a crucible and ashed overnight at 550 °C in a muffle furnace. The ash residue was digested in 5 ml of 6 M HCl at 50 °C for 30 minutes and filtered. After appropriate dilution, the concentration of nutrient elements were determined by direct aspiration on a calibrated simultaneous ICP spectrophotometer.

2.8. Statistical analysis

All measurements except $\delta^{15}N$ values were $\log_e$ transformed to reduce inequality of variance in the raw data. As a result of the differences in environmental conditions between plants grown on tables and in chambers (e.g. 1.5°C temperature difference), the data from plants grown on tables and those in chambers were analysed separately. Because the number of plants per replicate table or chamber were sometimes unequal,

a REML (residual maximum likelihood) variance component analysis (Genstat 1993) was used to statistically test differences on each species. Here, the Wald χ^2 statistic generated by REML tests for significant differences between UV-B effects. In this study, the UV-B levels were inserted in the fixed model and plants per table or chamber in the random model. Differences exceeding twice the standard error were used to separate significantly different treatment means at $P \leq 0.05$. This is based on the fact that for a normal distribution from REML estimates, the 5% two sided critical value is two. Correlation coefficients and the student's t-test method were used to test statistical relationship between parameters.

CHAPTER 3

EFFECTS OF ULTRAVIOLET-B RADIATION ON PLANT GROWTH, SYMBIOTIC FUNCTION AND CONCENTRATION OF METABOLITES IN THREE TROPICAL GRAIN LEGUMES

3.1 Introduction

Increases in UV-B radiation (280-315 nm) have been recorded in both southern and northern hemispheres (McKenzie *et al.* 1999; Seckmeyer *et al.* 1994), but with uncertain consequences for crop plants (Fiscus and Booker 1995). This rise in UV-B radiation is due to depletion of the stratospheric ozone layer, the principal attenuator of solar UV-B radiation, which is reported to have adverse effects on photosynthesis and growth of many plant species (Rozema *et al.* 1997; Teramura and Sullivan 1994). As a defence mechanism, plants accumulate phenylpropanoid compounds, such as flavonoids and anthocyanins, in response to UV-B radiation (Bieza and Lois 2001; Mazza *et al.* 2000; Rozema *et al.* 1997). But these molecules also serve as plant signals to symbiotic bacteria in the Rhizobiaceae (Cullimore and Dénarié 2003; Phillips 2000; Dakora and Phillips 1996), and their accumulation in root tissues is known to promote nodule formation (Pinto *et al.* 2002; Muofhe and Dakora 1999). Recent studies have also shown direct evidence of the induction of NADP-malic enzymes by UV-B radiation in leaves, stems and roots of three bean cultivars (Casati and Andreo 2001; Pinto *et al.* 1999), and in leaves of maize (Drincovich *et al.* 1998). From these results, it was suggested that NADP-malic enzyme plays an active role in plant defence responses against UV-B, possibly by providing NADPH for lignin and flavonoid biosynthesis (Casati and Andreo 2001). The accumulation of flavonoids and/or anthocyanins in plant organs such as roots could influence nodulation and symbiotic function in N_2 -fixing legumes exposed to elevated UV-B radiation. The aim of this study was to assess the effects of UV-B radiation on plant growth, symbiotic function and concentration of metabolites in three tropical food grain legumes.

3.2 Experimental

Seeds *V. unguiculata*, *G. max* and *P. vulgaris* were sown during mid-summer in pots (3 seeds per pot) containing sand and inoculated (see section 2.1). The pots were irrigated daily with equal volumes of water, and the seedlings later thinned to one per pot. Twice

every week, 400 mL of ½ strength N-free Hoagland's nutrient solution (Hewitt 1966) was supplied to each potted plant.

The experiment was carried out on 9 separate tables (3 replicates by three UV treatment) with banks of fluorescent sun lamps (Philips TL/12 40W UV-B, The Netherlands) for above ambient UV-B treatments, and 9 separate chambers. Six pots of a given species were placed on each table or chamber. Thus, there were three replicates of each UV treatment, with six subsamples within each replicate. This experiment compared two elevated levels of UV-B (32 and 62% above ambient) with a control (ambient) treatment, or below ambient (22% of ambient) with two controls, one for PAR control and the other for UV-A control. (see section 2.2 for details of the UV treatments).

Plants of *V. unguiculata*, *G. max* and *P. vulgaris* were harvested 65, 68 or 72 d respectively after germination, and separated into nodules, roots, stems, leaves and pods. At this time, *G. max* and *P. vulgaris* were at pod formation stage of growth, while *V. unguiculata* was still at flowering stage. Nodules were counted, and together with the other plant organs oven-dried at 60°C to a constant dry weight. After recording the dry weights, each sample was ground into a fine powder for analysis of N and metabolites

Concentrations of N in all plant organs and seeds of the parent material were measured as %N following the procedure outlined in section 2.4. Measurement of phenylpropanoids, and non-structural carbohydrates were done as indicated in sections 2.5 and 2.6 respectively. Ureides assay was done on xylem sap successfully collected from only *V. unguiculata*. At harvest plants were decapitated at the crown, and the exuding xylem sap collected and stored at –4°C prior to analysis. Ureide concentration in the xylem was assayed in sap samples as described by Dakora *et al.* (1992), and allantoin together with allantoic acid measured spectrophotometrically as the phenylhydrazone of glyoxylate (Trijbels & Vogels 1966). Data were analysed statistically using REML (residual maximum likelihood) variance component analysis (Genstat 1993) (see section 2.8 for details)

3.3 Results

3.3.1 *Above-ambient UV-B exposures*

Plant growth

Both the moderately (UV-B_{132}) and highly elevated (UV-B_{162}) UV-B radiation had no effect in all three species studied, whether on the basis of whole plant dry matter or the individual plant organs (Table 3.1).

Symbiotic performance

Similarly, nodule numbers, nodule dry matter, nodule size, N fixed $plant^{-1}$ and N content of different plant organs in all of the three species tested were not affected by the elevated UV-B (Table 3.1). However, plant exposure to moderately elevated UV-B decreased ($P \leq 0.05$) nodule activity in *G. max* (Table 3.1). Nitrogen concentration, measured as %N, was also reduced ($P \leq 0.05$) in roots of *G. max* plants grown under elevated UV-B. In *P. vulgaris*, %N of roots decreased with exposure to UV-B_{162}, while in leaves, it decreased when plants were exposed to UV-B_{132} (Table 3.1). In contrast, there was an increase ($P \leq 0.01$) in %N of stems and leaves of *V. unguiculata* plants grown under highly elevated UV-B (Table 3.1). Xylem concentrations of ureides in *V. unguiculata* plants were unaffected by the elevated exposures (Table 3.1).

Table 3.1 Effects of elevated UV-B radiation on nodulation, N_2 fixation, root metabolite concentrations and biomass accumulation of three tropical grain legumes. Significantly different means within species at *P ≤ 0.05, **P ≤ 0.01, in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{132} = 32% above ambient ultraviolet-B, UV-B_{162} = 62% above ambient. - = not determined.

	G. max				***P. vulgaris***				***V. unguiculata***			
	UV-B_{100}	UV-B_{132}	UV-B_{162}	Wald χ^2 statistic	UV-B_{100}	UV-B_{132}	UV-B_{162}	Wald χ^2 statistic	UV-B_{100}	UV-B_{132}	UV-B_{162}	Wald χ^2 statistic
Nodules												
Number plant^{-1}	189.6	194.9	157.6	2.30	162.6	177.9	153.1	0.68	122.0	94.3	130.9	1.55
Dry mass (g plant^{-1})	1.25	1.25	1.13	1.63	0.17	0.25	0.18	2.06	0.38	0.28	0.46	1.31
Size (mg nodule^{-1})	7.0	6.7	14.0	1.74	1.1	1.4	1.3	2.28	3.1	3.3	3.5	0.70
N concentration (%)	5.72	5.65	5.82	1.83	5.53	5.26	5.52	1.26	5.59	5.83	5.26	1.19
N content (mg N plant^{-1})	71.3	70.2	65.7	0.43	9.3	13.2	9.9	1.72	20.8	16.1	23.7	1.04
Activity (mg N fixed g^{-1} d^{-1})	**4.6a**	**4.0b**	**4.7a**	**6.23***	9.2	8.2	12.9	2.19	9.5	8.9	9.01	0.61
Roots												
Dry mass (g plant^{-1})	4.27	4.19	4.04	0.09	0.49	0.64	0.72	1.94	2.07	1.53	2.21	1.03
N concentration (%)	**2.00a**	**1.81b**	**1.78b**	**5.92***	**2.82a**	**3.04a**	**2.60b**	**7.11***	1.93	1.74	1.65	0.26
N content (mg N plant^{-1})	85.1	76.1	72.4	1.27	13.8	19.4	19.2	2.09	33.4	25.8	36.2	1.40
Flavonoids (Abs g^{-1})	48.6	56.1	58.3	1.28	66.1	63.0	65.7	0.61	33.5	34.6	37.2	0.10
Anthocyanins (Abs g^{-1})	0.52	0.65	0.68	0.07	0.82	0.95	1.04	0.70	0.46	0.53	0.47	0.86
Soluble sugars (mg g^{-1})	21.6	23.5	30.2	4.96	31.8	36.4	28.3	1.02	30.2	29.2	29.4	0.47
Starch (mg g^{-1} DM)	72.7	74.0	74.6	0.70	97.7	86.8	102.2	1.08	106.4	103.5	96.6	0.49
Stems												
Dry mass (mg plant^{-1})	3.64	3.29	3.44	1.66	0.48	0.71	0.77	1.02	1.66	1.18	1.92	1.22
N concentration (%)	2.53	2.46	2.54	0.76	2.37	2.13	2.32	5.57	**3.06b**	**3.00b**	**3.35a**	**12.25****
N content (mg N plant^{-1})	92.2	81.4	87.5	1.66	11.2	15.0	18.0	0.67	51.5	37.2	63.7	1.41
Ureides (µmol ml^{-1} sap)	-	-	-	-	-	-	-	-	16.8	22.0	18.3	1.28
Leaves												
Dry mass (g plant^{-1})	5.18	4.29	4.90	5.17	0.70	0.90	0.96	0.59	2.86.4	2.09	3.16	1.02
N concentration (%)	2.53	2.50	2.54	0.19	**4.13a**	**3.83b**	**4.20a**	**5.86***	**4.64b**	**4.74b**	**5.11a**	**6.12***
N content (mg N plant^{-1})	131.3	109.5	124.4	3.27	29.2	35.2	40.4	0.35	131.7	103.0	158.4	1.20
Pods												
Dry mass (g plant^{-1})	0.39	0.34	0.42	1.48	2.59	4.10	3.01	0.94	-	-	-	-
N concentration (%)	4.53	4.29	4.45	2.35	2.31	2.26	2.30	0.27	-	-	-	-
N content (mg N plant^{-1})	17.6	14.3	18.9	2.14	53.7	83.4	70.5	0.87	-	-	-	-
Whole plant												
Dry mass (g plant^{-1})	14.41	13.35	13.93	1.26	4.17	6.11	5.65	0.46	6.97	5.08	7.75	1.06
N fixed (mg plant^{-1})	388.4	342.4	359.8	1.68	101.0	146.8	141.8	0.36	232.0	176.9	276.7	1.26

Root metabolites and their correlation with symbiotic parameters

Root concentrations of flavonoid-like compounds (A_b: 300 nm), anthocynanins, soluble sugars and starch were unchanged in all the species tested under elevated UV-B radiation (Table 3.1). However, there were positive correlations ($P \leq 0.05$) between nodule N_2-fixing activity and the concentration of root flavonoid-like compounds, anthocyanins and starch in *G. max* (Table 3.2). In contrast, negative correlations ($P \leq 0.05$) were obtained between symbiotic parameters (nodule numbers, mass, size and nodule N_2-fixing activity) and concentration of plant metabolites (flavonoid-like compounds, anthocyanins, soluble sugars and starch) in both *V. unguicutata* and *P. vulgaris* (Table 3.2).

3.3.2 *Below-ambient UV-B exposures*

Plant growth

The sub-ambient UV-B_{22} exposure reduced ($P \leq 0.05$) total dry matter of *V. unguiculata* plants and their individual organs (Table 3.3) relative to both UV-A and PAR controls. Plants from UV-B_{22} treatment were 74 and 101% lower in biomass compared to UV-A and PAR controls, respectively. In contrast, the dry matter of whole plants and of individual organs in *G. max* and *P. vulgaris* were not affected by the below ambient UV-B radiation.

Symbiotic performance

Nodule numbers, nodule dry matter, N fixed plant^{-1} and N content of organs of *V. unguiculata* were decreased ($P \leq 0.05$) at UV-B_{22} relative to both PAR and UVA controls, but xylem concentration of ureides was unaltered (Table 3.3). However, in *G. max* and *P. vulgaris*, none of the symbiotic parameters was affected with plant exposure to below- ambient UV-B radiation (Table 3.3).

Table 3.2. Correlates of nodule numbers, mass, and activity against concentrations of metabolites and non-structural carbohydrates in roots of three tropical grain legumes cultivated under above-ambient UV-B exposures. Significant correlates at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 presented in bold type

Species/Parameter	Nodule numbers		Nodule mass		Nodule size		Nodule activity	
	r	t-statistic	r	t-statistic	r	t-statistic	r	t-statistic
Glycine max								
Root flavonoids	-0.06	$t_{1,36}$ = -0.38	0.04	$t_{1,36}$ = 0.22	0.07	$t_{1,36}$ = 0.43	**0.30**	**$t_{1,36}$ = 1.91***
Root anthocyanins	0.05	$t_{1,36}$ = 0.32	0.22	$t_{1,36}$ = 1.36	0.02	$t_{1,36}$ = 0.11	**0.38**	**$t_{1,36}$ = 2.50****
Root soluble sugars	-0.11	$t_{1,36}$ = -0.66	-0.13	$t_{1,36}$ = -0.72	0.07	$t_{1,36}$ = 0.41	0.13	$t_{1,36}$ = 0.80
Root starch	-0.12	$t_{1,36}$ = -0.70	0.03	$t_{1,36}$ = 0.21	0.12	$t_{1,36}$ = 0.74	**0.37**	**$t_{1,36}$ = 2.40***
Phaseolus vulgaris								
Root flavonoids	-0.15	$t_{1,26}$ = -0.77	-0.26	$t_{1,26}$ = -1.39	-0.19	$t_{1,26}$ = -0.98	-0.10	$t_{1,26}$ = -0.53
Root anthocyanins	-0.25	$t_{1,26}$ = -1.32	-0.13	$t_{1,26}$ = -0.66	0.25	$t_{1,26}$ = 1.29	0.07	$t_{1,26}$ = 0.35
Root soluble sugars	0.01	$t_{1,25}$ = 0.004	0.02	$t_{1,25}$ = 0.08	0.03	$t_{1,25}$ = 0.14	**-0.44**	**$t_{1,25}$ = 2.45***
Root starch	**-0.35**	**$t_{1,25}$ = -1.84***	**-0.64**	**$t_{1,25}$ = -4.18*****	**-0.65**	**$t_{1,25}$ = -4.33*****	0.20	$t_{1,25}$ = 0.16
Vigna unguiculata								
Root flavonoids	-0.12	$t_{1,36}$ = -0.70	**-0.53**	**$t_{1,36}$ = -3.70*****	**-0.58**	**$t_{1,36}$ = -4.22*****	0.07	$t_{1,36}$ = 0.45
Root anthocyanins	-0.04	$t_{1,41}$ = -0.26	**-0.42**	**$t_{1,41}$ = -2.94****	**-0.54**	**$t_{1,41}$ = -4.10*****	-0.23	$t_{1,41}$ = -1.49
Root soluble sugars	**-0.36**	**$t_{1,41}$ = -2.50****	**-0.45**	**$t_{1,41}$ = -3.21****	**-0.26**	**$t_{1,41}$ = -1.69***	-0.22	$t_{1,41}$ = -1.47
Root starch	**-0.30**	**$t_{1,41}$ = -1.99***	**-0.46**	**$t_{1,41}$ = -3.30****	**-0.33**	**$t_{1,41}$ = -2.27***	**-0.26**	**$t_{1,41}$ = -1.76***

Table 3.3. Effects of below ambient UV-B radiation on nodulation, N_2 fixation, root metabolite concentrations and biomass accumulation of three tropical grain legumes. Significantly different means within species at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 presented in bold type and separated by different letters. UV-B_{22} = 22% of ambient ultraviolet-B radiation, UV-$A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetic active radiation control. - = not determined.

	G. max				*P. vulgaris*				*V. unguiculata*			
	$PAR_{cont.}$	UV-$A_{cont.}$	UV-B_{22}	Wald χ^2 statistic	$PAR_{cont.}$	UV-$A_{cont.}$	UV-B_{22}	Wald χ^2 statistic	$PAR_{cont.}$	UV-$A_{cont.}$	UV-B_{22}	Wald χ^2 statistic
Nodules												
Number plant^{-1}	115.9	89.9	165.4	4.59	107.6	120.4	99.3	0.59	**104.0a**	**106.9a**	**68.1b**	**5.90***
Dry mass (g plant^{-1})	0.77	0.60	0.83	3.47	0.16	0.15	0.13	0.14	**0.46a**	**0.38a**	**0.27b**	**6.47***
Size (mg nodule^{-1})	6.6	9.6	5.6	2.22	1.5	1.4	1.3	0.60	4.6	3.9	3.6	3.05
N concentration (%)	5.73	6.07	5.62	1.93	3.23	3.51	4.80	3.06	6.85	7.01	6.41	1.23
N content (mg N plant^{-1})	43.9	35.4	46.8	3.14	5.1	5.7	5.6	0.77	**31.2a**	**26.6a**	**17.7b**	**6.72***
Activity (mg N fixed g^{-1} d^{-1})	7.1	6.3	6.8	1.54	19.9	17.8	12.9	1.35	13.0	13.2	12.7	1.75
Roots												
Dry mass (g plant^{-1})	2.45	1.67	2.31	3.05	0.88	0.73	0.65	0.89	**1.94a**	**2.17a**	**1.02b**	**10.60****
N concentration (%)	1.89	1.72	1.91	4.05	2.12	2.14	2.44	4.36	2.12	1.87	1.93	0.55
N content (mg N plant^{-1})	47.0	27.9	44.8	4.62	18.6	15.5	15.9	0.24	**41.3a**	**40.7a**	**16.8b**	**22.45*****
Flavonoids (Abs g^{-1})	69.3	76.0	70.5	0.21	61.4	61.5	71.7	1.64	36.9	42.6	41.1	1.09
Anthocyanins (Abs g^{-1})	0.88	0.82	0.76	0.15	0.84	1.05	1.01	1.47	0.42	0.38	0.43	1.71
Soluble sugars (mg g^{-1} DM)	**41.9a**	**23.2b**	**37.4a**	**24.34*****	20.4	13.7	18.9	1.90	28.0	22.7	29.7	5.51
Starch (mg g^{-1} DM)	**94.9a**	**86.9b**	**97.1a**	**9.30***	99.5	79.8	92.3	2.67	**81.3b**	**79.1b**	**104.3a**	**9.26***
Stems												
Dry mass (g plant^{-1})	3.02	2.15	3.39	3.61	1.07	0.69	0.58	1.19	**3.39a**	**2.48a**	**1.65b**	**7.56***
N concentration (%)	2.54	2.69	2.72	2.51	1.32	1.38	1.82	0.50	**3.38b**	**3.88a**	**3.55ab**	**10.21****
N content (mg N plant^{-1})	77.1	57.3	91.3	3.56	15.0	9.1	10.4	0.26	**113.6a**	**95.3a**	**56.7b**	**8.24***
Ureides (µmol ml^{-1} sap)	-	-	-	-	-	-	-	-	20.3	14.6	16.6	1.25
Leaves												
Dry mass (g plant^{-1})	4.06	2.91	4.28	3.38	1.12	1.09	0.76	2.65	**4.68a**	**4.02a**	**2.27b**	**8.77***
N concentration (%)	4.43	4.40	4.58	0.52	3.63	3.71	4.17	1.91	4.45	4.12	4.36	1.86
N content (mg N plant^{-1})	178.0	121.9	193.6	4.18	42.8	40.2	32.6	0.89	**206.4a**	**165.1a**	**103.1b**	**7.85***
Pods												
Dry mass (g plant^{-1})	0.73	0.52	0.50	2.36	3.79	3.71	1.61	0.44	-	-	-	-
N concentration (%)	3.88	4.17	4.12	2.48	2.58	3.18	2.85	4.18	-	-	-	-
N content (mg N plant^{-1})	29.0	20.8	21.4	2.28	91.6	118.1	47.9	0.36	-	-	-	-
Whole plant												
Dry mass (g plant^{-1})	11.03	7.85	11.30	3.24	7.01	6.31	3.74	1.45	**10.47a**	**9.05a**	**5.20b**	**8.87***
N fixed (mg plant^{-1})	365.79	254.11	386.84	3.82	156.8	117.9	96.2	1.25	**387.3a**	**322.4a**	**189.0b**	**8.76***

Root metabolites and their correlation with symbiotic parameters

Root concentrations of flavonoid-like compounds and anthocyanins were not altered by below-ambient radiation in all the three species tested (Table 3.3). However, exposing *V. unguiculata* plants to UV-B_{22} increased ($P \leq 0.05$) the concentration of starch in roots, while in *G. max*, it increased ($P \leq 0.05$) soluble sugar and starch concentrations relative to only the UV-A control.

There were negative correlations ($P \leq 0.05$) between root metabolites (anthocyanin and starch concentrations) and symbiotic parameters (nodule numbers, nodule dry weight and nodule size) in *V. unguiculata* (Table 4). Similarly, negative ($P \leq 0.05$) correlations were obtained between root metabolites (flavonoid-like compounds, anthocyanins and soluble sugars) and nodule N_2-fixing activity in *P. vulgaris*. This result contrasted with the positive correlation ($P \leq 0.05$) observed between root flavonoid concentration and nodule number in this species (Table 3.4). Marked positive correlations ($P \leq 0.05$) were also observed between root metabolites (soluble sugars and starch) and symbiotic traits (nodule numbers, nodule dry matter and nodule N_2-fixing activity) in *G. max* (Table 3.4).

3.4 Discussion

3.4.1 *Above-ambient UVB effects on plant growth and symbiotic function*

Exposing *V. unguiculata*, *P. vulgaris* and *G. max* to both moderately and highly elevated levels of UV-B radiation showed no effect on plant growth and individual organ development (Table 3.1). The lack of growth response observed here for the three legumes is compatible with the findings of similar studies conducted with other legumes (Mepsted *et al.* 1996; Stephen *et al.* 1999; Allen *et al.* 1998). Non-legume species such as sugar maple, rice, wheat and wild oat (Kim *et al.* 1996; Klironomos and Allen 1995; Barnes *et al.* 1988; Beyschlag *et al.* 1988) also showed no growth response when exposed to higher UV-B radiation. These findings however contrast with the reported reductions in plant biomass of *Vigna radiata* L. (Wilczek) cv. PS16, *Phaseolus mungo* L. (Hepper) cv. Mash-48 and *Glycine max* L. (Merr.) cv. Punjab 1 when grown

Table 3.4. Correlates of nodule numbers, mass, and activity against concentrations of metabolites and non-structural carbohydrates in roots of three tropical grain legumes cultivated under below ambient UV-B exposures. Significant correlates at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 presented in bold type.

Species/Parameter	Nodule numbers		Nodule mass		Nodule size		Nodule activity	
	r	t-statistic	r	t-statistic	r	t-statistic	r	t-statistic
Glycine max								
Root flavonoids	-0.18	$t_{1,28}$ = -0.96	**-0.32**	**$t_{1,28}$ = -1.80***	-0.11	$t_{1,28}$ = -0.60	0.09	$t_{1,28}$ = 0.48
Root anthocyanins	0.07	$t_{1,28}$ = 0.38	-0.03	$t_{1,28}$ = -0.16	-0.16	$t_{1,28}$ = -0.85	0.03	$t_{1,28}$ = 0.18
Root soluble sugars	**0.53**	**$t_{1,27}$ = 3.24****	**0.62**	**$t_{1,27}$ = 4.15*****	-0.08	$t_{1,27}$ = -0.44	0.09	$t_{1,27}$ = 0.49
Root starch	**0.49**	**$t_{1,28}$ = 2.94****	**0.42**	**$t_{1,28}$ = 2.48****	-0.28	$t_{1,28}$ = -1.53	**0.37**	**$t_{1,28}$ = 2.12***
Phaseolus vulgaris								
Root flavonoids	**0.40**	**$t_{1,18}$ = 1.85***	0.32	$t_{1,19}$ = 1.47	-0.10	$t_{1,18}$ = -0.42	**-0.65**	**$t_{1,19}$ = -3.72*****
Root anthocyanins	0.11	$t_{1,18}$ = 0.46	0.07	$t_{1,19}$ = 0.31	-0.059	$t_{1,18}$ = -0.25	**-0.53**	**$t_{1,19}$ = -2.70****
Root soluble sugars	-0.13	$t_{1,17}$ = -0.53	-0.08	$t_{1,18}$ = -0.33	0.08	$t_{1,17}$ = 0.33	**-0.40**	**$t_{1,18}$ = -1.84***
Root starch	0.15	$t_{1,17}$ = 0.61	0.17	$t_{1,18}$ = 0.73	0.08	$t_{1,17}$ = 0.32	-0.34	$t_{1,18}$ = -1.51
Vigna unguiculata								
Root flavonoids	-0.17	$t_{1,37}$ = -1.06	-0.25	$t_{1,37}$ = -1.52	-0.17	$t_{1,37}$ = -1.06	-0.16	$t_{1,37}$ = -0.96
Root anthocyanins	**-0.35**	**$t_{1,37}$ = -2.31***	**-0.31**	**$t_{1,37}$ = -1.97***	-0.03	$t_{1,37}$ = -0.23	-0.18	$t_{1,37}$ = -1.09
Root soluble sugars	-0.24	$t_{1,37}$ = -1.49	-0.18	$t_{1,37}$ = -1.13	0.03	$t_{1,37}$ = -0.19	-0.05	$t_{1,37}$ = -0.31
Root starch	**-0.43**	**$t_{1,37}$ = -2.89****	**-0.53**	**$t_{1,37}$ = -3.84*****	**-0.31**	**$t_{1,37}$ = -2.00***	-0.04	$t_{1,37}$ = -0.23

under elevated UV-B radiation (Singh 1996). These inconsistencies could be explained by either genotypic differences in UV-B sensitivity (Jansen *et al.* 1998), different environmental conditions under which plants were grown, and/or the intensity of UV-B supplementation (Fiscus and Booker 1995). In the study by Singh (1996), the total daily UV-B exposures (10.08 kJ m^{-2} d^{-1}) used for the three tropical legumes were similar to our moderately elevated UV-B (10.28 kJ m^{-2} d^{-1}) but smaller than our highly elevated UV-B (12.61 kj m^{-2} d^{-1}) yet were more damaging on plant growth. This is because the UV-B was applied intensely over only 2-h period a day compared to 8 h in our study to take into account the presence of ambient UV-B for almost the whole day. Consequently, the elevated UV-B was supplied at 4 times a greater intensity in the study by Singh (1996). Also, N and other mineral supplements such as farmyard manure were added to the soils in which those legumes were grown (Singh 1995), and both factors may have enhanced the UV-B sensitivity of plants. Marked UV-B effect has been reported for soybean grown under optimum P levels (Murali and Teramura 1985) and for N-fertilized, but not N-depleted, maize and cucumber plants (Hunt and McNeil 1998; Correira *et al.* 2000).

As with growth, the symbiotic response of the three test legumes was also unaffected by both the moderately and highly elevated UV-B levels, except for *G. max* where nodule activity declined. These findings contrast with the reduced nodule numbers, diameters and nitrogenase activities reported by Singh (1997) for closely related tropical legumes belonging to the tribe Phaseoleae. As indicated previously, these inconsistencies could either reflect genotypic differences in UV-B sensitivity, the intensity of UV-B radiation and/or the level of mineral supplementation.

Analysis of plant solutes such as xylem ureides in *V. unguiculata* also showed no effect of elevated UV-B on symbiotic N nutrition (Table 3.1). The concentration of metabolites such as flavonoid-like compounds and anthocyanins were similarly unaltered in roots of the three species studied. But because these molecules have been functionally linked to nodulation in *V. unguiculata*, *P. vulgaris* and *G. max* (Dakora 2000; Zhang and Smith 1997; Hungria and Phillips 1993), it is perhaps not surprising that symbiotic function was unaffected by elevated UV-B, since their concentrations in roots of the test species were also unaltered by elevated UV-B radiation. However, because the interaction involving *nod* signals occurs at the early stages of plant development, quantifying tissue flavonoid-like compounds at flowering and podding stages was probably inappropriate. Future studies should assess root flavonoids at early stage of seedling growth when the infection initials formed by root

nodule bacteria have just commenced. An additional problem with our approach was determining flavonoids after oven-drying as this could have degraded some components with high temperature.

3.4.2 *Below ambient UV-B effects on plant growth and symbiotic function*

Considering the lack of growth and symbiotic response by the three legume species to above ambient UV-B levels, the growth inhibition of *V. unguiculata* at sub-ambient UV-B in the chambers was difficult to interpret. Possible explanations could include the elevated air temperatures and altered spectral composition of light in the chambers. However, average maximum air temperatures in chambers (30. 7°C ± 0.5) were only slightly higher than background (29.1°C ± 1.1) and thus, the changes were minimal (range: 1.04 to 1.99 C). Notable is that the absolute maximum temperatures were well below those which these species encounter in their natural tropical environment during summer. Besides, it has been demonstrated that increased air temperatures can compensate for reduced growth in maize and sunflower under UV-B radiation (Mark and Tevini 1996). With regards to the altered light spectral composition in the chambers, mechanisms of interaction between UV-B radiation and other wavelengths of light have not yet been convincingly demonstrated (Allen *et al.* 1998). However, it was reported that both UV-A and PAR have moderating effects on UV-B damage by inducing photoreactivating process that effectively repairs DNA lesion resulting from UV-B radiation Jagger *et al.* (1969), and induction of phenylpropanoid biosynthesis of UV-B absorbing phenolics (Middleton and Teramura 1994). Studies of field grown *G. max* indicated significant UV-B decreases in total aboveground production and growth only when UV-A and PAR were reduced to less than half their flux in sunlight (Caldwell *et al.* 1994). Furthermore, photosynthetic inhibition proportional to the ratio of UV-B/(UV-A + PAR) has been shown to occur in Antarctic phytoplankton (Smith *et al.* 1992), although this may not be entirely applicable to growth inhibition in terrestrial plants. So, without sufficient UV-A or PAR, the UV-B damage can be considerably exacerbated.

Inside our chambers, PAR reductions (range 10.0 to 20.4%) were low, as a result the available PAR was enough to effectively mitigate UV-B damage. Furthermore, the level of PAR obtained here (1560; range 1480 to 1630 µmol m^{-2} s^{-1}) was, according to Saeed *et al.* (1999) and Horton (2000), sufficient for maximum photosynthetic performance of crop plants. In contrast, UV-A reductions (range: 66.2 to 80.6%) were quite substantial, and probably suggest ineffective UV-A mitigation of UV-B damage. For example, whole plant dry matter and that of individual organs and nodules in *V.*

unguiculuta plants showed no differences between the UV-A control and PAR control chambers but were reduced in the UV-B_{22} chamber. This response pattern could suggest UV-B induced inhibition of growth but no distinct mitigation by UV-A. Thus, UV-B effect in chambers was probably exacerbated because of the low amount of UV-A (19.4 – 33.8% of ambient) as discussed above. The differential growth response by *V. unguiculata* plants to below ambient UV-B radiation was similarly reflected in the amounts of N accumulated in different plant organs and total N fixed per plant, though not in nodule activity.

3.4.3 *Correlating the concentration of root metabolites with symbiotic function*

The results of correlation analysis between root metabolites and nodule number, mass, size and activity varied with species. The significant negative correlation between symbiotic traits (numbers, mass and size of nodules) and concentration of soluble sugars and starch in the roots of *P. vulgaris* and *V. unguiculata* plants (Table 3.2 and 3.4) suggests that there was probably a much lower demand for C by the few root nodules present or that UVB-induced compounds altered the C nutrition of developing nodules. In *G. max*, however, there was a positive correlation between numbers, dry matter and size of nodules and soluble sugars and starch in the roots, indicating a strong C investment in the nodulation of this species when exposed to below ambient UV-B.

Root concentration of flavonoid-like compounds, anthocyanins and starch also correlated positively with nodule activity in *G. max* plants exposed to elevated UV-B radiation (Table 3.2). Because these phenolic molecules serve as signals for nodule formation in legumes (Dakora 1994, 2000), the observed positive correlation probably indirectly indicates their link with the plant's total nodule population, which, in turn, defines the level of N_2-fixing activity. Similarly, flavonoids and anthocyanins can act as inhibitors of *nod* gene induction (Djordjevic *et al.* 1987), and their accumulation would therefore also correlate negatively with nodule function as observed for *P. vulgaris* plants under below ambient UV-B. But the negative correlation between root concentrations of flavonoid-like compounds/anthocyanins and nodule number/dry matter in *G. max* and *V. unguiculata* is probably the most direct indication of inhibitor effect on *nod* gene expression that decreased nodulation in plants exposed to below ambient UV-B.

Taken together, the results of this study show that neither plant growth nor symbiotic function was impaired by elevated UV-B radiation simulating 15 and 25% ozone

depletion in all the tropical grain legumes species studied. However, plant response to below ambient UV-B, and correlations between root metabolites and symbiotic performance varied with species.

CHAPTER 4

EFFECTS OF ULTRAVIOLET-B RADIATION ON NUTRIENT CONCENTRATION IN DIFFERENT ORGANS OF *VIGNA UNGUICULATA* AND *GLYCINE MAX*

4.1 Introduction

Whilst the impact of elevated UV-B radiation on plant physiology, morphology, growth and biomass have received considerable attention, little is known about UV-B effects on plant nutrition (Yue *et al.* 1998), and the few reports that are available show inconsistent results. For instance, Balakumar *et al.* (1999) reported UV-B induced alterations in the nitrate assimilation pathway of *V. unguiculata* where activity of nitrate and/or nitrite reductase were reduced with UV-B exposure. This was attributed to a reduction in the rate of photosynthate translocation between plant organs (Tingey *et al.* 1973). However, it has been reported that elevated UV-B radiation increased the concentration of all nutrients in soybean seedlings (Murali and Teramura 1985). Exposing 20 d old cowpea seedlings to elevated UV-B radiation also resulted in increased shoot concentrations of K, Ca, Na, Mg, Cd and Cu (Premkumar and Kulandaivelu 2001). Concentration of N and K increased in all plant organs of *Triticum aestivum* with UV-B exposure, but changes in levels of P, Mg, Fe and Zn varied with organ (Yue *et al.* 1998). In marine plankton communities, concentration of P consistently decreased, while that of N slightly increased with exposure to elevated UV-B radiation (Wängberg *et al.* 1998). In another study however, UV-B did not alter the concentration of C, N, lignin, and holocellulose in needles of *Pinus taeda* L. (Cybulski III *et al.* 2000).

Plant growth and tissue concentrations of nutrients are interdependent (Siddiqi and Glass 1982) and both depend on external concentration, uptake and translocation rate (Ingestad and Ågren 1992; Barrow 1977). Nutritionally, there is adequate zone of nutrient concentration below which growth is reduced (critical concentration), and above which growth is hindered (toxic concentration) (Marschner 1995). Furthermore, changes in concentration of nutrients in plants may strongly affect organ function and/or litter decay. For instance, reductions in N, Fe, and P content of plant organs, may hinder metabolic functions such as enzyme and photosynthetic pigment production, and energy requiring processes including N and P uptake (Pausch *et al.* 1996; Cromer *et al.* 1993; Abadia 1992). It is also shown that decomposition of organic matter is altered with nutrient concentration because the chemical composition of litter tissue determines its quality as an energy resource for decomposer organisms and can therefore influence its mass loss and nutrient release (Cybulski III *et al.* 2000; Coûteux *et al.* 1995).

When the concentrations of nutrients and water are adequate, nutrient acquisition depends on ion transport to the root and rate of absorption, which in turn depends on, among other factors, rates of chemical and biochemical reactions and production of photosynthates (de Varennes *et al.* 2002), processes which are reported to be vulnerable to elevated UV-B radiation (Rozema *et al.* 1997; Teramura and Sullivan 1994). With only limited number of experimental results, which are also inconsistent, it is uncertain how UV-B may alter the overall concentration of carbon, nutrients, and important secondary compounds in plant tissue. The objective of this study was to assess the effects of elevated UV-B radiation on concentration of macro- and micronutrients in different plant organs of *V. unguiculata* and *G. max*.

4.2 Experimental

Plant material from leaf, stem and root of *V. unguiculata* and *G. max* obtained from the UV-B exposure experiment above (Chapter 3) were analysed for macronutrients (N, P, K, Ca, Mg and Na) and micronutrients (Fe, Cu, Zn Mn and B). Nitrogen in plant samples was determined using a carlo Erba NA 1500 elemental analyser coupled to a Finniggan MAT 252 mass spectrometer via a Conflo II open-split device (Section 2.4). For the determination of the other nutrients, sub-samples were ashed at 550 °C in a muffle furnace. The ash was digested in 5 ml of 6 M HCl at 50 °C, and elements analysed on ICP spectrophotometer (Section 2.7)

4.3 Results

Concentration of macronutrients (N, P, K, Ca, Mg and Na) and micronutrients (Fe, Cu, Zn, Mn and B) in plant organs (leaf, stem, root and nodules) of *V. unguiculata* and *G. max* that did not show significant ($P \leq 0.05$) differences with plant exposure to UV-B radiation are not presented in the tables below.

4.3.1 Above-ambient UV-B exposures

Concentration of macronutrients

Exposing *V. unguiculata* to UV-B_{162} increased ($P \leq 0.05$) the concentration of leaf N and Ca, and stem N relative to ambient control and UV-B_{132} treatment (Table 4.1).

However, plants exposed to UV-B_{132} reduced ($P \leq 0.05$) concentration of stem P, and root P and K, in contrast to increased levels of nodule P and Ca relative to both ambient control and UV-B_{162} (Table 4.1). Magnesium and sodium were not altered in any organ of *V. unguiculata* exposed to elevated UV-B radiation (Table 4.1). With *G. max*, however, concentration of sodium increased in leaves and nodules with plant exposure to UV-B_{162}, but that of root N decreased with exposure to both levels of elevated UV-B (Table 4.1).

Concentration of micronutrients

The level of B in leaves of *V. unguiculata* increased ($P \leq 0.05$) with exposure to the highly elevated UV-B, in contrast to that in root and nodules which decreased ($P \leq 0.05$) (Table 4.2). Exposing *G. max* plants to UV-B_{132} increased ($P \leq 0.05$) concentration of Fe in leaves and B in stems relative to ambient UV-B control and elevated UV-B_{162} (Table 4.2). However, both levels of elevated UV-B radiation did not alter concentration of Cu, Zn and Mn in any organ of the test species (Table 4.2).

4.3.2 Below-ambient UV-B exposures

Concentration of macro-nutrient

Concentration of K and Ca in stems of *V. unguiculata* and *G. max*, and in leaves of the latter increased ($P \leq 0.05$) under below ambient UV-B_{22} relative to UV-A control but not PAR control (Table 4.3). This was in contrast to the concentration of Na in nodules of *V. unguiculata*, which decreased ($P \leq 0.05$) under UV-B_{22} relative to UV-A control but not PAR control (Table 4.3). However, N concentration in stems of *V. unguiculata* increased ($P \leq 0.01$) in plants under UV-A control relative to those under PAR control but not UV-B_{22} (Table 4.3). None of the macronutrient concentration in leaves and roots of *V. unguiculata*, and in roots and nodules of *G. max* changed with exposure to below ambient UV-B radiation (Table 4.3).

Concentration of micronutrients

Exposure of *V. unguiculata* to UV-B_{22} decreased ($P \leq 0.05$) concentration of Mn and/or Fe in nodules and roots relative to UV-A control, but not PAR control (Table 4.4). Concentration of Zn in stems of this species decreased ($P \leq 0.05$) relative to both UV-A and PAR controls, while in leaves it decreased ($P \leq 0.001$)

Table 4.1. Effects of elevated UV-B radiation on macronutrient concentration in different plant organs of *Vigna unguiculata* and *Glycine max*. Significantly different means at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{132} = 32% above ambient ultraviolet-B, UV-B_{162} = 62% above ambient.

Organ nutrient (mg/g)	Above ambient UV-B			Wald χ^2 Statistic (d.f. = 2)
	UV-B_{100}	UV-B_{132}	UV-B_{162}	
Vigna unguiculata				
Leaf				
N	**46.39b**	**47.42b**	**51.08a**	**6.12***
P	6.83	5.23	6.55	2.06
P	15.26	14.50	16.02	2.85
Ca	**9.84ab**	**9.73b**	**10.35a**	**6.10***
Mg	1.93	1.87	2.02	5.53
Na	0.22	0.25	0.30	3.87
Stem				
N	**30.62b**	**30.02b**	**33.47a**	**12.25****
P	**5.19a**	**4.57b**	**5.42a**	**7.65***
K	23.34	23.50	23.25	5.36
Ca	5.00	5.03	4.87	0.68
Mg	3.95	3.87	3.90	2.25
Na (µg/g)	720.50	763.67	789.50	3.02
Root				
N	19.29	17.39	16.48	0.26
P	**2.55a**	**2.03b**	**2.78a**	**6.21***
K	**17.38a**	**13.93b**	**16.92a**	**11.75****
Ca	1.83	1.60	1.72	2.75
Mg	1.88	1.90	1.82	3.29
Na	2.20	2.52	2.13	0.47
Nodule				
N	55.94	58.28	52.58	1.19
P	**5.68b**	**6.47a**	**5.53b**	**11.98****
K	19.10	23.07	18.77	1.00
Ca	**3.83b**	**14.57a**	**3.87b**	**11.20****
Mg	0.27	0.12	0.40	0.24
Na	0.62	5.22	5.32	2.21
Glycine max				
Leaf				
N	25.31	25.04	25.41	0.19
P	4.73	4.85	5.37	0.94
K	17.41	18.28	18.21	1.16
Ca	9.22	10.17	9.17	2.05
Mg	2.63	2.86	2.67	1.92
Na (µg/g)	**0.17b**	**0.15b**	**0.19a**	**8.31***
Stem				
N	25.31	44.62	25.41	0.76
P	3.51	3.68	3.08	2.03
K	14.45	17.18	14.81	3.12
Ca	7.08	7.76	6.58	1.92
Mg	3.18	3.42	3.28	2.20
Na (µg/g)	0.41	0.36	0.45	2.94
Root				
N	**19.99a**	**18.13b**	**17.78b**	**5.92***
P	2.88	3.61	2.67	1.71
K	12.56	14.15	11.67	4.68
Ca	1.38	1.23	1.11	0.23
Mg	1.25	1.23	0.89	0.05
Na	0.31	0.27	0.28	2.82
Nodule				
N	57.15	56.45	58.23	1.83
P	5.01	5.22	5.34	1.40
K	12.80	14.19	11.68	2.20
Ca	3.03	3.02	3.00	0.40
Mg	3.29	3.23	3.31	0.97
Na (µg/g)	**1.70b**	**1.39b**	**2.25a**	**9.97****

Table 4.2. Effects of elevated UV-B radiation on micronutrient concentration in different plant organs of *Vigna unguiculata* and *Glycine max*. Significantly different means at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{132} = 32% above ambient ultraviolet-B, UV-B_{162} = 62% above ambient.

Organ/Nutrient (μg/g)	Above ambient UV-B			Wald χ^2 Statistic
	UV-B_{100}	UV-B_{132}	UV-B_{162}	(d.f. = 2)
Vigna unguiculata				
Leaf				
Fe	140.58	151.58	139.38	0.24
Cu	2.220	2.380	2.148	3.15
Zn	14.02	14.01	15.78	1.25
Mn	25.15	30.01	27.42	3.04
B	**98.54b**	**127.62a**	**121.16a**	**8.38***
Stem				
Fe	74.11	69.33	81.96	1.43
Cu	2.458	2.300	2.223	2.54
Zn	20.77	24.10	22.84	1.87
Mn	16.97	19.38	17.32	2.09
B	38.00	36.43	43.60	2.20
Root				
Fe	2270.60	1947.79	2348.56	0.22
Cu	6.564	5.997	6.577	0.40
Zn	10.21	10.98	10.00.	0.58
Mn	19.21	17.54	21.32	0.06
B	**167.25a**	**72.55b**	**109.55b**	**9.40****
Nodule				
Fe	1181.10	1046.98	1368.29	0.11
Cu	5.181	5.093	5.572	1.27
Zn	19.28	25.30	19.27	1.66
Mn	7.995	2.163	7.765	2.15
B	**150.85a**	**69.75b**	**81.37b**	**9.92****
Glycine max				
Leaf				
Fe	**214.24b**	**239.62a**	**228.71ab**	**8.55***
Cu	3.350	2.961	2.809	0.11
Zn	18.77	20.53	18.93	2.07
Mn	23.08	26.21	22.42	0.79
B	89.98	91.04	90.09	0.26
Stem				
Fe	80.95	83.15	89.97	1.79
Cu	2.207	2.547	2.634	2.22
Zn	13.58	15.38	14.01	4.75
Mn	9.63	10.43	9.57	1.44
B	**30.68b**	**32.28a**	**30.39b**	**7.72***
Root				
Fe	2487.19	2226.62	2112.89	3.11
Cu	7.975	8.568	7.189	0.22
Zn	14.78	14.53	11.18	0.70
Mn	19.61	19.41	17.43	0.12
B	41.20	43.97	60.56	0.30
Nodule				
Fe	619.01	791.20	702.19	4.80
Cu	4.434	4.734	4.661	2.64
Zn	10.28	12.45	12.66	3.28
Mn	4.409	4.862	4.608	0.82
B	35.67	36.18	57.37	0.58

compared to only PAR control (Table 4.4). Iron level in stem of *V. unguiculata* exposed to UV-B_{22} increased ($P \leq 0.05$) relative to PAR but not UV-A control (Table 4.4). However, none of the micronutrients changed in organs of *G. max* following exposure to below-ambient UV-B radiation.

4.3.3 *Correlation between organ nutrient concentration and organ dry matter*

There were negative correlations ($P \leq 0.05$, 0.01 or 0.001) between concentration of Ca, Mg, Cu and B in leaves and leaf dry matter; Cu and stem dry matter; Fe and Mn and nodule dry matter of *V. unguiculata* (Table 4.5). However, Mg and root dry matter; K, Ca and nodule dry matter of this species showed positive correlations ($P \leq$ 0.05, 0.01). With *G. max*, concentration of P, K, Ca, Mg, Fe and Mn correlated negatively ($P \leq 0.01$ or 0.001) with leaf dry matter; K, Ca, Mg, Cu, Zn, and Mn with stem dry matter; and P, K, and Cu with root dry matter (Table 4.5). Under below ambient UV-B regime, fewer nutrient elements correlated significantly ($P \leq 0.05$, 0.01 or 0.001) with organ dry matter. For example, in *V. unguiculata*, only Mg, Fe correlated negatively with stem dry matter; and Na, Fe, Mn with nodule dry matter had significant negative correlation, but K, Mg and nodule dry matter correlated ($P \leq$ 0.05) positively (Table 4.6). With *G. max*, negative correlations ($P \leq 0.05$, 0.01 or 0.001) were observed between Fe and dry matter of all organs; Na and leaf dry matter; Mg and stem dry matter; and Ca, Zn, B and nodule dry matter. However, positive correlations ($P \leq 0.05$, 0.01) were shown between K, Na and stem dry matter, Mg and root dry matter; and Na and nodule dry matter (Table 4.6).

Table 4.3. Effects of below ambient UV-B radiation on macronutrient concentration in different plant organs of *Vigna unguiculata* and *Glycine max*. Significantly different means at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{22} = 22% of ambient ultraviolet-B radiation, UV-$A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control.

Organ nutrient (mg/g)	Below ambient UV-B			Wald χ^2 Statistic (d.f. = 2)
	$PAR_{cont.}$	UV-$A_{cont.}$	UV-B_{22}	
Vigna unguiculata				
Leaf				
N	44.45	41.21	43.58	1.86
P	3.77	3.70	5.25	0.27
P	15.75	13.23	19.55	1.01
Ca	13.45	10.53	9.05	1.85
Mg	1.95	1.68	1.65	3.89
Na	0.17	0.14	0.21	2.29
Stem				
N	**33.83b**	**38.75a**	**35.49ab**	**10.21***
P	3.27	3.58	3.72	1.10
K	**25.48a**	**19.20b**	**23.35a**	**11.19****
Ca	**6.25a**	**4.92b**	**5.84a**	**21.92*****
Mg	3.28	4.10	3.15	3.71
Na (µg/g)	0.47	0.48	0.46	1.09
Root				
N	21.23	18.67	19.34	0.55
P	2.37	2.13	1.90	2.53
K	20.83	16.20	13.45	5.66
Ca	1.17	1.10	3.95	1.46
Mg	1.47	1.57	1.35	3.38
Na	2.25	2.20	2.18	0.41
Nodule				
N	68.46	70.08	64.08	1.23
P	5.85	6.27	5.70	4.62
K	16.23	12.22	12.45	1.31
Ca	3.77	4.11	3.95	2.14
Mg	4.57	4.38	4.50	1.09
Na	**0.51b**	**0.65a**	**0.50b**	**6.18***
Glycine max				
Leaf				
N	44.31	44.01	45.82	0.52
P	3.10	2.68	4.03	2.97
K	**13.93a**	**9.90b**	**17.65a**	**9.44****
Ca	**9.80a**	**9.33b**	**13.16a**	**12.20****
Mg	3.13	2.88	3.26	0.49
Na (µg/g)	0.11	0.20	0.15	1.45
Stem				
N	25.43	26.93	27.05	2.51
P	2.43	2.15	2.67	3.17
K	**14.15a**	**10.23b**	**16.07a**	**6.20***
Ca	**7.75a**	**5.58b**	**8.61a**	**7.70***
Mg	2.58	2.70	2.92	0.14
Na (µg/g)	0.13	0.16	0.14	1.09
Root				
N	18.85	17.23	19.09	4.05
P	2.45	2.20	3.35	3.05
K	15.33	12.40	15.23	1.10
Ca	2.38	2.43	2.32	0.54
Mg	3.90	1.43	1.33	2.22
Na	1.77	2.40	1.64	2.25
Nodule				
N	57.26	60.70	56.19	1.93
P	5.35	6.13	5.38	0.39
K	12.48	10.75	12.48	1.61
Ca	0.358	0.325	0.355	0.25
Mg	3.75	3.80	3.77	0.33
Na (µg/g)	1.27	1.32	1.43	0.16

Table 4.4. Effects of below ambient UV-B radiation on micronutrient concentration in different plant organs of *Vigna unguiculata*. Significantly different means at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{22} = 22% of ambient ultraviolet-B radiation, UV-$A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control.

Organ nutrient (µg/g)	Below ambient UV-B			Wald χ^2 Statistic (d.f. = 2)
	$PAR_{cont.}$	UV-$A_{cont.}$	UV-B_{22}	
		Vigna unguiculata		
Leaf				
Fe	121.78	159.12	140.53	5.03
Cu	3.323	2.810	2.340	3.06
Zn	**15.65a**	**11.92b**	**12.73b**	**18.17*** **
Mn	18.06	22.31	16.92	2.00
B	97.74	120.65	88.05	1.92
Stem				
Fe	**60.58b**	**75.75a**	**99.53a**	**6.56***
Cu	3.070	2.555	3.740	1.79
Zn	**26.80a**	**21.84a**	**16.52b**	**7.46***
Mn	13.09	16.43	13.98	3.98
B	30.58	29.98	32.71	1.03
Root				
Fe	1703.51	1738.51	1379.74	1.20
Cu	6.62	5.67	6.66	1.37
Zn	15.24	9.11	15.48	1.42
Mn	**11.89ab**	**15.26a**	**8.79b**	**7.22***
B	110.56	106.53	97.79	0.56
Nodule				
Fe	**1577.23ab**	**2078.75a**	**835.11b**	**6.15***
Cu	6.868	6.865	4.675	2.91
Zn	21.29	18.80	18.12	0.89
Mn	**5.327ab**	**7.972a**	**1.715b**	**9.20***
B	90.12	60.30	65.85	2.87
		Glycine max		
Leaf				
Fe	219.22	214.45	209.58	0.85
Cu	3.610	4.810	4.439	4.30
Zn	17.71	17.24	17.49	0.15
Mn	17.96	18.23	18.56	0.10
B	63.43	80.13	73.19	1.40
Stem				
Fe	60.43	61.52	74.00	0.29
Cu	2.572	2.405	3.005	0.31
Zn	10.86	10.71	10.28	0.20
Mn	4.883	3.980	5.922	2.86
B	26.23	27.61	29.56	5.04
Root				
Fe	1771.55	1794.61	1074.89	0.85
Cu	6.918	8.713	5.695	4.22
Zn	10.87	13.74	9.05	1.69
Mn	14.58	13.44	10.65	0.56
B	61.78	36.30	50.11	2.85
Nodule				
Fe	820.81	898.95	813.68	0.48
Cu	5.388	6.048	5.590	2.49
Zn	12.07	12.74	11.28	1.69
Mn	3.793	4.118	3.981	0.56
B	39.29	45.53	33.21	0.62

Table 4.5. Correlation between individual organ dry matter and nutrient elements of *V. unguiculata* and *G. max* exposed to elevated UV-B radiation. Significant correlations at *P ≤ 0.05, **P ≤ 0.01, *** P ≤ 0.001 presented in bold type.

Nutrient	Leaf dry matter		Stem dry matter		Root dry matter		Nodule dry matter	
	r	t-statistic	r	t-statistic	r	t-statistic	r	t-statistic
V. unguiculata								
K	0.15	$t_{1,32} = 0.84$	0.36	$t_{1,28} = 1.98$	-0.03	$t_{1,27} = -0.15$	**0.53**	**$t_{1,23} = 2.95^{**}$**
Ca	**-0.42**	**$t_{1,32} = -2.56^{*}$**	-0.24	$t_{1,28} = -1.30$	-0.02	$t_{1,27} = -0.13$	**0.43**	**$t_{1,23} = 2.22^{*}$**
Mg	**-0.40**	**$t_{1,32} = -2.46^{*}$**	-0.01	$t_{1,28} = -0.07$	**0.45**	**$t_{1,27} = 2.55^{*}$**	0.31	$t_{1,23} = 1.55$
Fe	0.19	$t_{1,32} = 1.09$	-0.32	$t_{1,28} = -1.77$	-0.06	$t_{1,27} = -0.30$	**-0.46**	**$t_{1,23} = -2.43^{*}$**
Cu	**-0.50**	**$t_{1,32} = -3.19^{**}$**	**-0.63**	**$t_{1,28} = -4.17^{***}$**	-0.27	$t_{1,27} = -1.44$	-0.39	$t_{1,23} = -1.97$
Mn	-0.21	$t_{1,32} = -1.20$	0.35	$t_{1,28} = 1.93$	0.15	$t_{1,27} = 0.79$	**-0.46**	**$t_{1,23} = -2.42^{*}$**
B	**-0.46**	**$t_{1,32} = 2.86^{**}$**	-0.15	$t_{1,28} = -0.93$	-0.16	$t_{1,27} = -0.84$	-0.17	$t_{1,23} = -0.82$
G. max								
N	**0.50**	**$t_{1,37} = 3.42^{**}$**	0.30	$t_{1,37} = 1.92$	0.20	$t_{1,34} = 1.17$	0.01	$t_{1,37} = 0.08$
P	**-0.46**	**$t_{1,37} = -3.14^{**}$**	-0.20	$t_{1,37} = -1.23$	**-0.44**	**$t_{1,34} = -2.84^{**}$**	-0.09	$t_{1,37} = -0.53$
K	**-0.58**	**$t_{1,37} = -4.30^{***}$**	**-0.67**	**$t_{1,37} = -5.40^{***}$**	**-0.42**	**$t_{1,34} = -2.69^{*}$**	-0.23	$t_{1,37} = -1.43$
Ca	**-0.53**	**$t_{1,37} = -3.76^{***}$**	**-0.41**	**$t_{1,37} = -2.70^{*}$**	-0.06	$t_{1,34} = -0.33$	-0.18	$t_{1,37} = -1.08$
Mg	**-0.46**	**$t_{1,37} = -3.08^{**}$**	**-0.35**	**$t_{1,37} = -2.27^{*}$**	-0.08	$t_{1,34} = -0.48$	-0.11	$t_{1,37} = -0.64$
Fe	**-0.47**	**$t_{1,37} = -3.17^{**}$**	-0.06	$t_{1,37} = -0.34$	0.21	$t_{1,34} = 1.24$	0.18	$t_{1,37} = 1.09$
Cu	-0.04	$t_{1,37} = -0.21$	**-0.49**	**$t_{1,37} = -3.35^{**}$**	**-0.43**	**$t_{1,34} = -2.72^{*}$**	-0.31	$t_{1,37} = -1.95$
Zn	-0.47	$t_{1,37} = -3.22$	**-0.38**	**$t_{1,37} = -2.44^{*}$**	-0.07	$t_{1,34} = -0.43$	-0.23	$t_{1,37} = -1.42$
Mn	**-0.57**	**$t_{1,37} = -4.12^{***}$**	**-0.53**	**$t_{1,37} = -3.74^{***}$**	0.10	$t_{1,34} = 0.58$	-0.17	$t_{1,37} = -1.06$

Table 4.6. Correlation between individual organ dry matter and nutrient elements of *V. unguiculata* and *G. max* exposed to sub-ambient UV-B radiation. Significant correlations at *P ≤ 0.05, **P ≤ 0.01, *** P ≤ 0.001 presented in bold type.

Nutrient	Leaf dry matter		Stem dry matter		Root dry matter		Nodule dry matter	
	R	t-statistic	r	t-statistic	r	t-statistic	r	t-statistic
V. unguiculata								
N	-0.09	$t_{1,34} = -0.50$	**-0.40**	**$t_{1,36} = -2.59$***	-0.16	$t_{1,36} = -0.95$	-0.13	$t_{1,17} = -0.54$
K	-0.04	$t_{1,34} = -0.26$	-0.03	$t_{1,36} = -0.18$	-0.14	$t_{1,36} = -0.86$	**0.51**	**$t_{1,17} = 2.37$***
Mg	-0.004	$t_{1,34} = -0.23$	**-0.35**	**$t_{1,36} = -2.23$***	0.02	$t_{1,36} = 0.13$	**0.53**	**$t_{1,17} = 2.51$***
Na	0.08	$t_{1,34} = 0.45$	-0.28	$t_{1,36} = -1.72$	-0.09	$t_{1,36} = -0.51$	**-0.54**	**$t_{1,17} = -2.59$***
Fe	0.11	$t_{1,34} = 0.62$	**-0.48**	**$t_{1,36} = -3.21$****	0.19	$t_{1,36} = 1.17$	**-0.57**	**$t_{1,17} = -2.80$***
Mn	-0.26	$t_{1,34} = -0.57$	-0.31	$t_{1,36} = -1.99$	0.30	$t_{1,36} = 1.86$	**-0.54**	**$t_{1,17} = -2.58$***
G. max								
N	**-0.60**	**$t_{1,30} = -4.08$*****	0.05	$t_{1,27} = 0.24$	0.11	$t_{1,26} = 0.57$	-0.16	$t_{1,30} = -0.85$
K	0.27	$t_{1,30} = 1.55$	**0.54**	**$t_{1,27} = 3.28$****	0.34	$t_{1,26} = 1.82$	0.18	$t_{1,30} = 0.98$
Ca	0.29	$t_{1,30} = 1.65$	-0.13	$t_{1,27} = -0.67$	-0.15	$t_{1,26} = -0.75$	**-0.38**	**$t_{1,30} = -2.19$***
Mg	0.23	$t_{1,30} = 1.25$	**-0.67**	**$t_{1,27} = -4.63$*****	**0.43**	**$t_{1,26} = 2.38$***	-0.23	$t_{1,30} = -1.24$
Na	**-0.72**	**$t_{1,30} = -5.54$*****	**0.53**	**$t_{1,27} = 3.17$****	-0.10	$t_{1,26} = -0.50$	**0.53**	**$t_{1,30} = 3.34$****
Fe	**-0.53**	**$t_{1,30} = -3.36$****	**-0.51**	**$t_{1,27} = -3.02$****	**-0.41**	**$t_{1,26} = -2.25$***	**-0.48**	**$t_{1,30} = -2.94$****
Zn	-0.05	$t_{1,30} = -0.27$	-0.27	$t_{1,27} = -1.43$	0.36	$t_{1,26} = 1.91$	**-0.39**	**$t_{1,30} = -2.25$***
B	-0.39	$t_{1,30} = -2.23$*	0.02	$t_{1,27} = 0.09$	-0.35	$t_{1,26} = -1.85$	**-0.48**	**$t_{1,30} = -2.96$****

4.4 Discussion

4.4.1 *Above-ambient UVB effects on nutrient concentration*

Exposing plants of *V. unguiculata* and *G. max* to elevated UV-B radiation altered the concentration of P, Ca, K, Na, Fe and B in tissues. However, the extent of these changes varied with species, plant organ, and level of UV-B radiation. Under supra-ambient UV-B radiation, for example, leaf Ca, stem P, root P and root K were significantly decreased by *V. unguiculata* exposure to UV-B_{132} relative to UV-B_{162} or control (Table 4.1). Of the micro-nutrients, only B showed decreased concentration in roots and nodules of *V. unguiculata* with elevated UV-B radiation relative to the control. In leaves, however, B levels were significantly increased by elevated UV-B compared to the control (Table 4.1). In *G. max*, leaf and nodule Na increased by exposure to UV-B_{162} relative to UV-B_{132} or the control, where as leaf Fe and stem B increased by exposure to UV-B_{132}. The decreases in tissue concentration of minerals ought to affect cell function and plant growth in the two legume species as it is known, for example, that P is a constituent of macromolecular structures such as nucleic acids, DNA and RNA (Marschner 1995) and plays a major role in energy transfer as phosphorylated sugars and organic acids. Thus a decrease in the concentration of P in plant cells could lead to low rates of RUBP, NADPH and ATP regeneration, reduced cell division (Cromer et al. 1993) and consequently decreased plant growth (Le Bot et al. 1998). But this was not the case in these plants (Chapter 3). Similarly, K is the dominant counter-ion for the negative charges in cells, contributes markedly to cell turgor pressure and is associated with opening and closure of stomata (Maathuis and Sanders 1996) needed for CO_2 intake for photosynthesis and plant growth. In symbiotic terms, B has been shown to alter accumulation of phenolic nod gene inducers, decrease nodule numbers, and reduce the amounts of N fixed in legumes (Muofhe and Dakora 1999). Thus a lowering of its concentration in legumes should affect symbiotic function and N_2 fixation, but again that was not observed in earlier studies (Chapter 3), suggesting that the decrease in mineral composition of organs was related to allocation and not overall balance of minerals required for growth. Similarly, the increase in concentration of Fe in leaves of *G max* (Table 4.2) and stems of V. unguiculata (Table 4.4) with UV-B exposure possibly due to increasing photoreduction and therefore increasing mobility (Pushnik et al. 1987, Jolley et al.

1987), did not also affect plant growth nor symbiotic function. However, because some UV-B effects are subtle and can accumulate over several generations of UV-B exposure (Musil et al. 1999), these changes in the concentrations of P, K, or B in roots or nodules could negatively impact on future generations of UV-B exposed plants. Additionally, the rate of litter decomposititon could be altered with the observed changes in nutrient concentration (Cybulski III et al. 2000), resulting in altered nutrient release from soil organic matter. This, in turn, could significantly impact on nutrient cycling at the ecosystem level.

The differences in mineral concentration of various plant parts with elevated UV-B exposure observed in this study are consistent with reports by Premkumar and Kulandaivelu (2001), Larson *et al.* (1998) and Yue *et al.* (1988), and could be due to effects on nutrient uptake or changes in metabolic pathways (Yue *et al.* 1988). However, because adequate quantities of nutrients were supplied in this study (Hewitt 1966), and there were no correlations between nutrient concentrations and organ dry matter (Table 4.5), the observed changes in tissue mineral concentrations may be due to shifts in allocation pattern between organs for meeting specific demands, or to problems associated with nutrient transportation between organs (Ambler et al. 1975), or difficulties in uptake assimilation into plant tissue (Braune and Döhler 1994; Tyagi et al. 1992). Although the mechanisms for creating such effects remain unknown, plant exposure to elevated UV-B could affect nutrient transportation in roots and thus tissue concentrations. Besides the decreases in tissue levels of major minerals, there was an increase in the concentration of some nutrients such as P and Ca in nodules and B in leaves of *V. unguiculata*, just as leaf Fe increased in *G. max* plants exposed to elevated UV-B relative to control. Such increases of nutrient elements have been observed for soybean seedlings exposed to enhanced UV-B radiation (Murali and Teramura 1995), and was associated with changes in biomass production and/or allocation.

4.4.2 Below ambient UV-B effects on nutrient concentration

The concentrations of Ca and K in stems of both *V. unguiculata* and *G. max*, and in leaves of the latter, were significantly lower under UV-A control compared to UV-B_{22} or PAR control. However, with *V. unguiculata* but not *G. max*, stem Zn concentration, root Mn and nodule Fe and Mn were reduced under UV-B_{22} relative to UV-A or PAR

control (Table 3). As with elevated UV-B radiation, the mechanisms underlying the decrease of Zn, Mn and Fe in stems, roots and nodules of *V. unguiculata* under UV-B_{22} relative to UV-A and PAR controls are not clearly understood, just as those which up-regulate Ca and K in shoot (leaves and stems) of both *G. max* and *V. unguiculata* under UV-A control relative to UV-B_{22} and PAR control are still unknown.

Although the biomass of individual organs and whole plants changed in *V. unguiculata* with exposure to below ambient UV-B (Chapter 3), there were no positive correlations between nutrient concentration and organ dry matter (Table 6). Thus, variations in nutrient levels are less likely to be associated with concentration or dilution effect due to changes in organ biomass, but rather with problems of nutrient transport and incorporation (Braune and Döhler 1994; Tyagi *et al.* 1992; Ambler *et al.* 1975) or allocation patterns. The negative correlation between Fe and dry matter in all organs of both species exposed to below ambient UV-B could suggest that its concentration was beyond sufficiency.

In UV-B studies, both UV-A and PAR are reported to have moderating effects on UV-B damage through induction of photo-reactivating processes that repair DNA lesions resulting from UV-B radiation (Jagger *et al.* 1969), and/or by stimulation of biosynthesis of UV-B absorbing phenolics (Middleton and Teramura 1994). However, there are also reports that ambient UV-A can reduced biomass production in plants such as *Lactuca sativa* (lettuce) and *Cucumis sativus* (cucumber) over and above that caused by ambient UV-B radiation (Krizek *et al.* 1998; Krizek *et al.* 1997). Therefore, the changes in nutrient concentration between UV-A control and PAR control (Tables 4.3 and 4.4) can be attributed to the presence of UV-B and higher levels of UV-A radiation in the UV-A control chamber.

In conclusion, our results show that elevated UV-B radiation altered concentration of P, K, B, Na and Fe in different plant organs without affecting growth and symbiotic function of the two test species.

CHAPTER 5

RESPONSE OF PURELY SYMBIOTIC AND NO_3-FED NODULATED PLANTS OF *LUPINUS LUTEUS* AND *VICIA ATROPURPUREA* TO ULTRAVIOLET-B RADIATION

5.1 Introduction

Available data indicate that the ultraviolet-B (UV-B, 290 - 315) radiation reaching the earth's surface has increased (Madronich *et al.* 1998) as a result of depletion of stratospheric ozone, the principal attenuator of UV-B (Mckenzie *et al.* 1999). Several reports have shown that UV-B radiation can depress photosynthesis, reduce plant growth and reproduction, and alter species competitive interactions (Jansen *et al.* 1998; Rozema *et al.* 1997; Teramura and Sullivan 1994). However, plant growth and photosynthetic response to elevated UV-B radiation can be species-specific (Yuan *et al.* 2000; Jansen *et al.* 1998) and is also modified by environmental and nutritional factors (Correia *et al.* 2000; Murali and Teramura 1985). As a result, extrapolation of data between, or even within, species can be difficult.

Sensitivity of plants to UV-B radiation is reported to be affected by the N concentration of the growth medium. This is because a marked effect was observed on N-fertilized, but not N-depleted maize and cucumber plants following exposure to elevated UV-B (Correia *et al.* 2000; Hunt and McNeil 1998). Although N fertilization is rarely practised due to the N inhibition of nodulation and N_2 fixation, legumes grown on N-poor soils can become N-limited before the onset of N_2 fixation (Ma *et al.* 1997). Thus supplementing legumes with mineral N can be beneficial for plant growth and yields. Plant response to environmental stress can also differ depending on their source of nutrients. In one study, pea plants relying entirely on N_2-fixation were more tolerant of salt stress compared to their N-fertilized counterparts (Del Pilar Cordovilla *et al.* 1999). It remains to be known whether legume species depending entirely on symbiotic N_2 fixation would be more tolerant, or susceptible to elevated UV-B relative to their N-fertilized counterparts. The aim of this study was to assess the effects of UV-B radiation on growth, symbiotic function and concentration of metabolites of *Lupinus luteus* and *Vicia atropurpurea* plants either depending entirely on symbiotic N_2 fixation for their nutrition or fed 2 mM NO_3.

5.2 Experimental

Lupinus luteus and *V. atropurpurea*, important folder and cover crops grown in the Western Cape of South Africa, were used in this study. Seeds of *L. luteus* and *V.*

atropurpurea were sown in potted sand and inoculated with *Bradyrhizobium lupini* and *Rhizobium leguminosarum* biovar *viciae* respectively (see. section 2.1). There were 12 separate tables with banks of fluorescent sun lamps (Philips TL/12 40W UV-B, The Netherlands) and 12 chambers constructed of differentially UV-transmitting clear perspex inter-dispersed in an open area in the Kirstenbosch National Botanical Gardens, Cape Town (see section 2.2). Each table or chamber had 4 pots with plants of each test species. For N treatment, 2 of the 4 pots received 2 mM NO_3 while plants in the other 2 pots relied entirely on symbiotic N_2 fixation for their N nutrition. All pots were irrigated with equal volumes of water, and the seedlings later thinned out to two plants per pot. Immediately at seedling emergence, 400 mL of ½ strength N-free Hoagland's nutrient solution (Hewitt 1966) was supplied twice weekly to the purely symbiotic plants and the same volume made up to 2 mM NO_3 applied to N-fed plants.

There were two elevated UV-B treatments (UV-B_{137} and UV-B_{173}) and one control each replicated four times (see section 2.2.1 for details of UV-B supplementation). Measured UV-B exposures over the winter growing period of plants averaged 99.4% (range: 90.4% to 106.4%) of background in the UV-B_{100} control, 143.1% (range 132.7% to 149.9%) of background in the UV-B_{137} treatment and 176.8% (range: 159.6 to 193.38%) of background in the UV-B_{173} treatment. Under Sub-ambient UV-B conditions, there was one UV-B treatment (UV-B_{22}), and two controls; one for photosynthetically active radiation (PAR control) and the other for UV-A (UV-A control) radiation (section 2.2.2), each replicated four times. Average maximum daily air temperatures in the chambers (24.4 ± 0.60°C) and background (23.3 ± 0.56°C) were recorded.

Plants of *L. luteus* and *V. atropurpurea* were harvested at 108 and 128 d respectively after germination, and separated into nodules, roots, stems, leaves and pods. At this time, *L. luteus was* at pod formation stage of growth, while *V. atropurpurea* was still at flowering stage. The plant organs were oven-dried, weighed and ground into a fine powder for analysis of N and metabolites (section 2.3).

Analysis of N in tissues, measurement of tissue concentration of flavonoid-like compounds and non-structural carbohydrates were performed as indicated in sections 2.4, 2.5, and 2.6 respectively. Data were analysed statistically using REML (residual maximum likelihood) variance component analysis (Genstat 1993) (see section 2.8).

5.3 Results

5.3.1 Above-ambient UV-B exposures

Plant growth

Exposing *L. luteus* and *V. atropurpurea* plants to elevated UV-B radiation did not change plant total biomass or the dry matter of individual organs (Table 5.1). Also, feeding the plants with NO_3 did not alter organ or total dry matter in both species except for leaves of *L. luteus* which increased with NO_3 supply (Table 5.1).

Although the interactions between UV-B and NO_3 application were not significant ($P \leq 0.05$) for any plant growth parameter of *V. atropurpurea* (Table 5.1), leaf and total dry matter of NO_3-fed *L. luteus* plants increased ($P \leq 0.05$) with exposure to UV-B_{173} relative to the control (UV-B_{100}). Total dry matter of purely symbiotic *L. luteus* plants also increased ($P \leq 0.05$) relative to the UV-B_{137}, but not the control (Table 5.1).

Symbiotic performance

Symbiotic components such as nodule dry matter and N fixed per plant were not altered in both *L. luteus* and *V. atropurpurea* plants with exposure to elevated UV-B radiation (Table 5.2). However, the concentration of N in *L. luteus* stems and leaves, unlike other organs was significantly decreased ($P \leq 0.05$) by UV-B_{173} relative to only UV-B_{137}. Supplying 2 mM NO_3 to *L. luteus* plants significantly reduced %N in nodules relative to their purely symbiotic counterparts (Table 5.2). Root %N of *V. atropurpurea* was also reduced by NO_3 application, in contrast to nodule dry matter which increased significantly ($P \leq 0.05$) (Table 5.2).

The interactions between UV-B and NO_3 feeding was significant ($P \leq 0.05$) for only nodule dry matter and total N of *L. luteus* (Table 5.2) as both parameters increased

($P \leq 0.05$) in NO_3-fed, but not purely symbiotic plants with exposure to elevated UV-B radiation. A comparison of NO_3 vs. symbiotic nutrition at each level of UV-B did not

alter total or organ dry matter in both species (Appendix 1). However, in *V. atropurpurea* there was an increase ($P \leq 0.05$) in nodule dry matter with NO_3 feeding at both UV-B_{100} and UV-B_{137}, but not UV-B_{173} (Appendix 2). In contrast, NO_3 nutrition decreased nodule dry matter in *L. luteus* relative to purely symbiotic plants at UV-B_{100} (Appendix 2).

Tissue concentrations of flavonoids and metabolites

Exposure of *L. luteus* plants to the highly elevated UV-B level significantly ($P \leq 0.05$) increased the concentration of flavonoid-like compounds (A_b: 300 nm) in leaves (Table 5.3), but not in those of *V. atropurpurea* (Tables 5.3). With N supply, the concentrations of flavonoid-like compounds and anthocyanins decreased markedly ($P \leq 0.05$) in the leaves of both *L. luteus* and *V. atropurpurea*, and also in the roots of the latter (Table 5.3). The levels of flavonoid-like compounds, anthocyanins and soluble sugars were similarly decreased ($P \leq 0.05$) in roots of *V. atropurpurea* plants supplied with N (Table 5.3).

As with their plant growth parameters, UV-B and NO_3 interacted significantly ($P \leq 0.05$) in only *L. luteus* when it comes to root anthocyanin and concentration of flavonoid-like compounds in leaves, but not in *V. atropurpurea* (Table 5.3). Relative to control and UV-B_{137}, the highly elevated UV-B radiation significantly increased the concentration of root anthocyanins in purely symbiotic, but not in NO_3-fed, plants of *L. luteus* (Table 5.3). Conversely only concentration of flavonoid-like compounds in leaves of NO_3-fed, but not purely symbiotic plants increased with UV-B exposure of this species. Applying NO_3 to *L. luteus* plants grown at each UV-B level decreased root anthocyanins at UV-B_{173} and concentration of flavonoid-like compounds in leaves at UV-B_{137} but not at the other UV-B level (Appendix 3).

Table 5.1. Effects of elevated UV-B radiation on organ or total dry matter accumulation of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea*. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{137} = 37% above ambient ultraviolet-B, UV-B_{173} = 73% above ambient. - = not determined.

N source/ UV-B treatment		Plant growth (g plant^{-1}) Root dry matter	Stem dry matter	Leaf dry matter	Pod dry matter	Total dry matter
				Lupinus luteus		
Main effects:						
	UV-B_{100}	2.83	3.85	2.36	0.34	9.81
	UV-B_{137}	2.77	3.39	2.21	0.28	9.01
	UV-B_{173}	3.23	4.47	2.62	0.42	11.20
	Symbiotic N	2.84	3.73	**2.26b**	0.37	9.63
	NO_3-N	3.05	4.08	**2.53a**	0.32	10.44
Interactions:						
Symbiotic N	UV-B_{100}	2.85	3.85	2.32a	0.43	**9.93ab**
	UV-B_{137}	2.69	3.16	2.08a	0.26	**8.58b**
	UV-B_{173}	2.99	4.17	2.35a	0.46	**10.38a**
NO_3-N	UV-B_{100}	2.78	3.83	**2.36b**	0.26	**9.58b**
	UV-B_{137}	3.13	3.88	**2.46ab**	0.33	**10.18ab**
	UV-B_{173}	3.67	5.01	**2.98a**	0.43	**12.59a**
Wald χ^2 statistic						
UV-B (d.f.=2)		1.75	4.66	2.69	3.88	3.32
N (d.f.=1)		1.83	1.68	**4.72***	0.46	1.85
UV-BxN (d.f.=2)		4.29	3.42	**5.91***	1.96	**6.09***
				Vicia atropurpurea		
Main effects:						
	UV-B_{100}	7.13	4.62	6.13	-	18.20
	UV-B_{137}	6.26	4.99	5.88	-	17.37
	UV-B_{173}	6.02	4.52	5.50	-	16.27
	Symbiotic N	6.93	5.01	5.63	-	17.74
	NO_3-N	5.96	4.61	6.04	-	16.76
Interactions:						
Symbiotic N	UV-B_{100}	7.93	5.33	5.80	-	19.22
	UV-B_{137}	6.55	5.20	5.31	-	17.23
	UV-B_{173}	6.30	4.50	5.74	-	16.77
NO_3-N	UV-B_{100}	6.21	4.35	6.17	-	17.04
	UV-B_{137}	6.23	4.92	6.39	-	17.88
	UV-B_{173}	5.73	4.54	5.26	-	15.78
Wald χ^2 statistic						
UV-B (d.f.=2)		1.40	0.54	0.57	-	0.77
N (d.f.=1)		1.88	0.69	0.33	-	0.54
UV-BxN (d.f.=2)		0.35	0.63	0.92	-	0.51

Table 5.2. Effects of elevated UV-B radiation on symbiotic parameters of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea*. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. $UV\text{-}B_{100}$ = ambient ultraviolet-B radiation, $UV\text{-}B_{137}$ = 37% above ambient ultraviolet-B, $UV\text{-}B_{173}$ = 73% above ambient. - = not determined.

N source/UV-B treatment		Nodule dry matter (g plant^{-1})	Nodule %N	Root %N	Stem %N	Leaf %N	Pod %N	Total N content (mg plant^{-1})
				Lupinus luteus				
Main effects:								
	$UV\text{-}B_{100}$	433.07	7.21	1.30	**2.23ab**	**5.48ab**	5.17	300.91
	$UV\text{-}B_{137}$	386.53	7.20	1.46	**2.39a**	**5.64a**	4.98	295.76
	$UV\text{-}B_{173}$	460.86	7.24	1.57	**2.16b**	**5.23b**	5.00	343.60
	Symbiotic N	454.05	**7.35a**	1.51	2.35	5.51	5.14	307.93
	NO_3-N	399.59	**7.08b**	1.38	2.18	5.40	4.95	320.53
Interactions:								
Symbiotic N	$UV\text{-}B_{100}$	0.49a	7.39	1.38	2.15	5.52	5.29	309.9a
	$UV\text{-}B_{137}$	0.40a	7.23	1.56	2.56	5.77	5.22	287.1a
	$UV\text{-}B_{173}$	0.44a	7.40	1.69	2.28	5.14	4.87	318.6a
NO_3-N	$UV\text{-}B_{100}$	**0.35b**	7.06	1.26	2.27	5.40	4.96	**286.3b**
	$UV\text{-}B_{137}$	**0.38ab**	7.16	1.47	2.14	5.45	4.71	**305.9b**
	$UV\text{-}B_{173}$	**0.50a**	7.05	1.50	2.04	5.33	5.16	**372.2a**
Wald χ^2 statistic:								
UV-B (d.f.=2)		1.65	0.29	3.63	**4.71***	**6.26***	0.70	3.03
N (d.f.=1)		1.52	**3.82***	1.63	3.51	0.35	0.40	0.24
UV-BxN (d.f.=2)		**11.24****	0.24	0.42	5.42	2.44	2.73	**6.98***
				Vicia atropurpurea				
Main effects:								
	$UV\text{-}B_{100}$	0.23	4.43	2.49	2.43	3.80	-	525.7
	$UV\text{-}B_{137}$	0.24	4.91	2.55	2.48	3.96	-	524.2
	$UV\text{-}B_{173}$	0.24	4.75	2.62	2.43	3.97	-	495.5
	Symbiotic N	**0.17b**	4.64	**2.79a**	2.47	3.87	-	532.1
	NO_3-N	**0.30a**	4.76	**2.31b**	2.44	3.96	-	496.9
Interactions:								
Symbiotic N	$UV\text{-}B_{100}$	0.16a	4.29	2.55	2.31	3.83	-	542.6
	$UV\text{-}B_{137}$	0.10a	4.73	1.87	2.48	3.84	-	522.4
	$UV\text{-}B_{173}$	0.23a	4.89	2.93	2.56	3.93	-	531.5
NO_3-N	$UV\text{-}B_{100}$	0.31a	4.58	2.42	2.52	3.78	-	506.4
	$UV\text{-}B_{137}$	0.34a	5.08	2.25	2.48	4.15	-	539.6
	$UV\text{-}B_{173}$	0.25a	4.61	2.30	2.30	4.02	-	459.5
Wald χ^2 statistic:								
UV-B (d.f.=2)		0.30	2.49	0.50	0.31	1.73	-	0.30
N (d.f.=1)		**9.04****	0.23	**6.79***	0.14	0.60	-	0.94
UV-BxN (d.f.=2)		**6.53***	1.22	1.28	3.29	0.68	-	0.37

5.3.2 Below *ambient UV-B exposures*

Plant growth

Growing *L. luteus* and *V. atropurpurea* plants in chambers under sub-ambient UV-B radiation did not alter overall plant growth or organ development (Table 5.4). However, supplementing the nodulated plants with NO_3 reduced ($P \leq 0.05$) dry matter accumulation in stems, leaves, pods and whole plants of the two species (Table 5.4). But root dry matter was reduced in only *V. atropurpurea* with NO_3 supply (Table 5.4). There was also a significant ($P \leq 0.05$) interaction between sub-ambient UV-B and N source (Table 5.4). Stem, leaf and total dry matter of purely symbiotic *V. atropurpurea* plants increased ($P \leq 0.05$) with plant exposure to UV-B_{22} but not visible or UV-A controls. In contrast, NO_3 supply decreased ($P \leq 0.05$) stem dry matter of *V. atropurpurea* plants receiving UV-B_{22} and UV-A relative to visible control (Table 5.4).

Symbiotic performance

Nodule dry matter, %N, N fixed, and nodule N_2-fixing activity were all unaltered in both *L. luteus* and *V. atropurpurea* plants exposed to sub-ambient UV-B radiation (Tables 5.5). However, supplying NO_3 to roots of *L. luteus* increased ($P \leq 0.05$) the concentration of N in leaves and stems, but reduced ($P \leq 0.05$) nodule dry matter, %N in nodules, and total plant N (Table 5.5). With *V. atropurpurea*, however, the NO_3 application significantly increased ($P \leq 0.05$) %N in stems and leaves (Table 5.5).

Table 5.3. Effects of elevated UV-B radiation on concentration of flavonoids, anthocyanins and non-structural carbohydrates in roots or leaves of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea*. Significantly different means within N source treatment at $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{137} = 37% above ambient ultraviolet-B, UV-B_{173} = 73% above ambient.

N source/UV-B treatment		Root flavonoids (Abs g^{-1})	Root anthocyanins (Abs g^{-1} DM)	Root soluble sugars (mg g^{-1} DM)	Root starch (mg g^{-1} DM)	leaf flavonoids (Abs g^{-1} DM)	Leaf anthocyanins (Abs g^{-1} DM)
				Lupinus luteus			
Main effects:							
	UV-B_{100}	93.12b	0.60	3.98	71.74	194.13	**1.22b**
	UV-B_{137}	96.29b	0.68	3.57	71.12	191.47	**1.20b**
	UV-B_{173}	145.62a	0.96	4.12	75.75	206.12	**1.65a**
	Symbiotic N	118.45a	0.83	4.70	70.33	**203.25a**	**1.49a**
	NO_3-N	104.91a	0.67	3.05	75.41	**191.23b**	**1.16b**
Interactions:							
Symbiotic N	UV-B_{100}	107.2	**0.65b**	4.43	72.44	204.1a	1.29
	UV-B_{137}	111.0	**0.67b**	4.20	66.96	210.0a	1.34
	UV-B_{173}	152.2	**1.15a**	5.41	73.63	205.2a	1.74
NO_3-N	UV-B_{100}	89.2	0.53a	3.52	73.66	**189.7ab**	1.05
	UV-B_{137}	91.5	0.70a	2.52	76.50	**175.7b**	0.68
	UV-B_{173}	144.7	0.78a	2.52	79.11	**207.9a**	1.66
Wald χ^2 statistic:							
UV-B (d.f.=2)		**9.03***	1.05	0.70	0.15	2.26	**6.71***
N (d.f.=1)		1.97	2.22	2.23	1.67	**3.74***	**4.84***
UV-BxN (d.f.=2)		0.99	**6.15***	1.43	0.33	**5.93***	4.07
				Vicia atropurpurea			
Main effect:							
	UV-B_{100}	59.30	1.08	7.46	60.33	134.59	2.96
	UV-B_{137}	58.43	0.99	7.68	59.39	137.51	3.42
	UV-B_{173}	55.01	1.15	8.37	64.95	124.62	2.20
	Symbiotic N	**67.45a**	**1.36a**	**8.80a**	62.14	**139.64a**	**4.09a**
	NO_3-N	**47.25b**	**0.79b**	**6.87b**	60.97	**124.84b**	**1.56b**
Interactions:							
Symbiotic N	UV-B_{100}	68.71	1.42	7.92	60.45	141.5	4.26
	UV-B_{137}	63.87	1.18	8.21	58.44	137.5	4.99
	UV-B_{173}	69.77	1.48	10.28	67.52	139.9	3.02
NO_3-N	UV-B_{100}	53.35	0.77	7.39	57.95	123.0	1.43
	UV-B_{137}	52.23	0.86	6.65	61.40	138.7	2.00
	UV-B_{173}	40.25	0.81	6.46	62.38	109.4	1.17
Wald χ^2 statistic:							
UV-B (d.f.=2)		0.32	1.49	0.59	1.01	3.30	2.91
N (d.f.=1)		**19.98*****	**42.08*****	**6.73***	0.22	**6.42***	**38.15*****
UV-BxN (d.f.=2)		2.57	1.24	2.76	0.93	4.65	0.17

Table 5.4. Effects of below-ambient UV-B radiation on organ or total dry matter accumulation of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea*. Significantly different means within N source treatment at $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ in bold type and separated by different letters. $UV\text{-}B_{22}$ = 22% of ambient ultraviolet-B radiation, $UV\text{-}A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control. - = not determined.

N source/ UV-B treatment		Plant growth (g plant^{-1}) Root dry matter	Stem dry matter	Leaf dry matter	Pod dry matter	Total dry matter
				Lupinus luteus		
Main effects:						
	$Visible_{cont.}$	2.24	3.18	2.33	1.28	9.68
	$UV\text{-}A_{cont.}$	2.51	3.63	2.67	1.35	10.94
	$UV\text{-}B_{22}$	2.25	3.23	2.51	1.29	10.83
	Symbiotic N	2.43	**3.87a**	**2.86a**	**1.64a**	**11.80a**
	NO_3-N	2.22	**2.80b**	**2.14b**	**0.95b**	**9.03b**
Interactions:						
Symbiotic N	$Visible_{cont.}$	2.23a	3.57	2.47	1.51	10.19
	$UV\text{-}A_{cont.}$	2.75a	4.75	3.52	2.05	13.61
	$UV\text{-}B_{22}$	2.71a	4.05	3.16	1.45	11.84
NO_3-N	$Visible_{cont.}$	2.40a	3.07	2.37	1.07	9.24
	$UV\text{-}A_{cont.}$	2.66a	3.12	2.33	0.66	9.09
	$UV\text{-}B_{22}$	2.312a	3.02	2.51	1.04	9.21
Wald χ^2 statistic:						
UV-B (d.f.=2)		0.19	0.33	0.06	0.87	0.31
N (d.f.=1)		1.45	**10.42****	**4.95***	**4.58***	**13.47*****
UV-BxN (d.f.=2)		**6.75***	5.11	5.19	2.68	5.53
				Vicia atropurpurea		
Main effects:						
	$Visible_{cont.}$	3.06	4.60	4.91	-	12.79
	$UV\text{-}A_{cont.}$	2.61	3.63	3.82	-	10.23
	$UV\text{-}B_{22}$	2.68	7.02	7.53	-	17.46
	Symbiotic N	**3.10a**	**5.91a**	**6.19a**	-	**15.41a**
	NO_3-N	**2.41b**	**4.09b**	**4.49b**	-	**11.18b**
Interactions:						
Symbiotic N	$Visible_{cont.}$	3.88	**5.01b**	**5.57b**	-	**14.64b**
	$UV\text{-}A_{cont.}$	3.34	**4.65b**	**4.88b**	-	**13.09b**
	$UV\text{-}B_{22}$	2.64	**8.16a**	**8.38a**	-	**19.46a**
NO_3-N	$Visible_{cont.}$	2.40	**4.20a**	4.52a	-	11.75a
	$UV\text{-}A_{cont.}$	2.04	**2.76b**	2.80a	-	7.85a
	$UV\text{-}B_{22}$	1.66	**2.11b**	2.62a	-	6.48a
Wald χ^2 statistic						
UV-B (d.f.=2)		1.04	0.07	0.24	-	0.07
N (d.f.=1)		**6.20***	**7.04****	**4.06***	-	**7.54****
UV-BxN (d.f.=2)		0.12	**13.06****	**8.84***	-	**9.04***

The interaction between sub-ambient UV-B and N sources was significant for only %N in leaves of *L. luteus* (Table 5.5), in that exposure to UV-B_{22} of purely symbiotic plants increased ($P \leq 0.05$) leaf %N relative to UV-A but not visible controls. With *V. atropurpurea*, UV-B_{22} increased %N in roots of purely symbiotic plants, but decreased that of NO_3-fed plants relative to visible but not UV-A controls (Table 5.5).

Tissue concentrations of flavonoids and metabolites

The concentration of flavonoid-like compounds, anthocyanins, soluble sugars and starch were unchanged in root and leaf organs of both *L. luteus* and *V. atropurpurea* plants exposed to sub-ambient UV-B radiation (Table 5.6). However, supplying NO_3 to *L. luteus* plants markedly reduced ($P \leq 0.05$) the concentration of flavonoid-like compounds, soluble sugars and starch in leaves and roots as well as anthocyanins in roots only (Table 5.6). The concentration of flavonoid-like compounds in leaves of *V. atropurpurea* plants was also reduced ($P \leq 0.05$) with NO_3 supply (Table 5.6). As shown for other parameters, there was a significant interaction between sub-ambient UV-B and N nutrition. In NO_3-fed, but not purely symbiotic plants exposure to UV-B_{22} decreased ($P \leq 0.05$) anthocyanins and soluble sugars in roots of *L. luteus* and *V. atropurpurea* respectively when compared to visible control (Table 5.6). Comparing NO_3 and symbiotic N nutrition at each level of sub-ambient UV-B showed a large reduction ($P \leq 0.05$) in root anthocyanins of NO_3-fed *L. luteus* plants grown at UV-B_{22} (Appendix 6). With *V. atropurpurea*, NO_3 supply more than halved root soluble sugars of plants grown under UV-A relative to their symbiotic counterparts (Appendix 6).

5.3.3 *Correlating root metabolites with symbiotic parameters and leaf flavonoids*

There was a positive correlation ($P \leq 0.05$) between root concentrations of flavonoid-like compounds or anthocyanins and nodule dry matter in *L. luteus* grown under elevated UV-B radiation (Table 5.7). Positive correlations ($P \leq 0.05$) were also observed between flavonoid-like compounds or anthocyanins in the roots of *L. luteus* (Table 5.7). In V. *atropurpurea*, however, the concentration of root anthocyanins correlated negatively with nodule dry matter. The levels of non-structural carbohydrates in roots of both species were unrelated to symbiotic parameters (Table 5.7).

Table 5.5. Effects of below-ambient UV-B radiation on symbiotic parameters of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea*. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{22} = 22% of ambient ultraviolet-B radiation, UV-$A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control. - = not determined.

N source/UV-B treatment		Nodule dry matter (g plant^{-1})	Nodule %N	Root %N	Stem %N	Leaf %N	Pod %N	Total N content (mg plant^{-1})
				Lupinus luteus				
Main effects:								
	$Visible_{cont.}$	0.35	7.54	1.02	2.11	4.72	2.97	270.96
	UV-$A_{cont.}$	0.39	7.76	1.16	1.99	4.58	3.26	303.56
	UV-B_{22}	0.36	7.79	1.15	2.18	4.71	2.94	305.17
	Symbiotic N	**0.45a**	**8.04a**	1.11	**2.00b**	**4.43b**	3.06	**327.34a**
	NO_3-N	**0.29b**	**7.34b**	1.11	**2.19a**	**4.91a**	3.05	**255.19b**
Interactions:								
Symbiotic N	$Visible_{cont.}$	0.41	**7.76**	1.07	2.06	**4.59a**	2.93	288.3
	UV-$A_{cont.}$	0.55	7.99	1.26	1.83	**4.10b**	3.34	370.5
	UV-B_{22}	0.47	8.30	1.07	2.06	**4.45a**	2.97	329.1
NO_3-N	$Visible_{cont.}$	0.32	**7.22**	0.96	2.14	**4.78a**	3.02	255.8
	UV-$A_{cont.}$	0.31	7.56	1.18	2.11	4.95a	3.22	257.8
	UV-B_{22}	0.32	7.46	1.22	2.20	4.73a	2.90	266.8
Wald χ^2 statistic:								
UV-B (d.f.=2)		0.23	2.07	1.56	4.18	0.95	0.89	0.42
N (d.f.=1)		**14.27*****	**17.20*****	0.03	**6.53***	**29.20*****	0.03	**12.05****
UV-BxN (d.f.=2)		4.49	2.32	0.89	1.82	**11.05****	1.22	4.42
				Vicia atropurpurea				
Main effects:								
	$Visible_{cont.}$	0.22	6.16	2.49	2.40	4.39	-	403.98
	UV-$A_{cont.}$	0.18	6.40	2.47	2.66	4.30	-	337.45
	UV-B_{22}	0.22	6.43	2.33	2.49	4.13	-	524.31
	Symbiotic N	0.22	6.18	2.40	**2.43b**	**4.06b**	-	469.88
	NO_3-N	0.19	6.51	2.47	**2.70a**	**4.52a**	-	363.84
Interactions:								
Symbiotic N	$Visible_{cont.}$	0.18	5.83	**1.97b**	2.42	4.21	-	464.0a
	UV-$A_{cont.}$	0.22	6.55	**2.70a**	2.37	3.97	-	419.3a
	UV-B_{22}	0.27	6.46	**2.39ab**	2.41	4.12	-	574.0a
NO_3-N	$Visible_{cont.}$	0.22	6.23	**2.76a**	2.45	4.32	-	389.2a
	UV-$A_{cont.}$	0.13	6.41	**2.24ab**	2.99	4.75	-	275.9a
	UV-B_{22}	0.19	6.73	**2.19b**	2.62	4.45	-	240.8a
Wald χ^2 statistic:								
UV-B (d.f.=2)		0.22	0.37	0.37	0.44	0.24	-	0.07
N (d.f.=1)		0.46	1.28	0.28	**5.73***	**6.51***	-	2.50
UV-BxN (d.f.=2)		1.33	0.69	**8.69***	4.81	5.44	-	**5.86***

Table 5.6. Effects of below-ambient UV-B radiation on concentration of flavonoids, anthocyanins and non-structural carbohydrates in roots or leaves of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea*. Significantly different means within N source treatment at $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ in bold type and separated by different letters. UV-B_{22} = 22% of ambient ultraviolet-B radiation, UV-$A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control.

N source/UV-B treatment		Root flavonoids (Abs g^{-1})	Root anthocyanins (Abs g^{-1} DM)	Root soluble sugars (mg g^{-1} DM)	Root starch (mg g^{-1} DM)	leaf flavonoids (Abs g^{-1} DM)	Leaf anthocyanins (Abs g^{-1} DM)
				Lupinus luteus			
Main effects:							
	$Visible_{cont.}$	78.20	0.69	2.70	82.26	209.20	0.73
	UV-$A_{cont.}$	82.38	0.70	2.89	70.93	224.42	0.81
	UV-B_{22}	75.99	0.66	3.61	83.79	22611	0.62
	Symbiotic N	**86.30a**	**0.76a**	**4.43**	**84.59a**	**229.79a**	0.72
	NO_3-N	**71.10b**	**0.60b**	**1.72b**	**74.09b**	**209.42b**	0.72
Interactions:							
Symbiotic N	$Visible_{cont.}$	82.36	0.70a	4.06	90.69	215.1	0.58
	UV-$A_{cont.}$	92.99	0.85a	3.99	78.20	232.6	0.90
	UV-B_{22}	88.61	0.83a	5.36	85.76	237.8	0.63
NO_3-N	$Visible_{cont.}$	75.85	**0.71a**	1.39	73.94	204.4	0.82
	UV-$A_{cont.}$	75.72	**0.62ab**	1.73	65.30	204.1	0.74
	UV-B_{22}	65.53	**0.58b**	0.86	70.16	212.2	0.76
Wald χ^2 statistic:							
UV-B (d.f.=2)		1.65	0.48	0.58	3.44	2.73	1.16
N (d.f.=1)		**13.89*****	**8.57****	**18.10*****	**7.13****	**4.50***	0.27
UV-BxN (d.f.=2)		3.90	**9.29***	0.36	1.11	0.61	2.12
				Vicia atropurpurea			
Main effects:							
	$Visible_{cont.}$	65.08	0.91	12.37	72.53	103.35	0.79
	UV-$A_{cont.}$	53.16	0.89	11.70	72.54	103.17	0.85
	UV-B_{22}	57.98	0.83	7.12	69.89	104.61	1.17
	Symbiotic N	62.75	0.95	11.71	72.43	107.56	**1.212**
	NO_3-N	53.94	0.79	9.54	70.70	99.06	**0.59b**
Interactions:							
Symbiotic N	$Visible_{cont.}$	61.72	1.04	7.93a	66.11	109.7	1.40
	UV-$A_{cont.}$	57.47	0.96	16.37a	74.66	122.1	1.23
	UV-B_{22}	68.92	1.00.	11.05a	79.76	105.1	1.16
NO_3-N	$Visible_{cont.}$	68.44	0.96	**17.14a**	74.90	102.1	0.44
	UV-$A_{cont.}$	48.14	0.81	**6.25b**	70.06	88.4	0.44
	UV-B_{22}	45.23	0.67	**5.92b**	64.76	104.2	1.00
Wald χ^2 statistic:							
UV-B (d.f.=2)		2.38	0.13	3.04	0.37	0.20	0.74
N (d.f.=1)		1.08	1.85	0.85	0.02	1.01	**9.79****
UV-BxN (d.f.=2)		2.05	0.72	**6.26***	1.72	2.07	1.45

With below ambient UV-B exposure, nodule dry matter was positively correlated ($P \leq 0.05$) with both flavonoid-like compounds and anthocyanin concentration in roots of *L. luteus* (Table 5.7). However, there were no significant ($P \leq 0.05$) correlations between root metabolits and any symbiotic parameters following exposure of *V. atropurpurea* plants to below ambient UV-B (Table 5.7).

5.4 Discussion

5.4.1 *Above-ambient UV-B effects on plant growth and symbiotic function*

Whether measured on the basis of whole plants, or individual organs, plant growth response was unaltered following exposure of *L. luteus* and *V. atropurpurea* to elevated UV-B radiation simulating 15 and 25% ozone depletion. Similarly, there was no change in nodulation and N_2 fixation of both *L. luteus* and *V. atropurpurea* with exposure to elevated UV-B radiation (Table 5.2). This lack of growth and symbiotic response of *L. luteus* and *V. atropurpurea* to elevated UV-B radiation is consistent with data obtained for *Vicia faba* (Al-Oudat *et al.* 1998) and *Pisum sativum* plants (Allen *et al.* 1999). Our findings however differ from those of Singh (1996) and Hofmann *et al.* (2001) who found major reductions in plant dry matter of *Vigna radiata*, *Phaseolus mungo*, *Glycine max*, *Trifolium repens*, and cultivars of *Pisum sativum* grown under elevated UV-B radiation. Unlike our data, studies of Singh (1997) and Van de Staaij *et al.* (1999) found large decreases in nodule numbers, nodule mass, nodule diameter and nitrogenase activity in symbiotic legumes exposed to elevated UV-B. Although these variations in growth and symbiotic response of nodulated legumes to elevated UV-B could be attributed to genotypic differences in plant sensitivity (Jansen *et al.* 1998), and/or nutritional status of the plant (Correia *et al.* 2000; Hunt and McNeil 1998; Murali and Teramura 1985), it appears that the intensity of UV-B radiation applied could be a major factor (Fiscus and Booker 1995). As discussed elsewhere (Chapter 3), although the total daily UV-B exposure used by Singh (1996) was below our highly elevated UV-B, though similar to our moderately elevated UV-B, the reduction in plant growth and symbiotic function was huge, and would be more likely due to the high intensity of UV-B applied over a short 2-h time period compared to the 8-h spread in our study.

Table 5.7. Correlations of nodule mass, leaf flavonoids and leaf anthocyanins with concentrations of metabolites and non-structural carbohydrates in roots of *Lupinus luteus* and *Vicia atropurpurea* plants exposed to above and below ambient UV-B radiation Significant correlations at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 presented in bold type. - = not determined

Species/Parameter	Nodule mass		Leaf flavonoids		Leaf anthocyanins	
	r	t-statistic	r	t-statistic	r	t-statistic
Above ambient UV-B exposures						
Lupinus luteus						
Root flavonoids	**0.51**	**$t_{1,34}$ = 3.44****	0.22	$t_{1,34}$ = 1.29	0.03	$t_{1,34}$ = 0.18
Root anthocyanins	**0.36**	**$t_{1,34}$ = 2.19***	0.24	$t_{1,34}$ = 1.40	-0.002	$t_{1,34}$ = -0.01
Root soluble sugars	0.29	$t_{1,34}$ = 1.76	-	-	-	-
Root starch	0.15	$t_{1,34}$ = 0.89	-	-	-	-
Vicia atropurpurea						
Root flavonoids	-0.27	$t_{1,45}$ = -1.80	**0.53**	**$t_{1,43}$ = 4.01*****	0.29	$t_{1,43}$ = 1.96
Root anthocyanins	**-0.36**	**$t_{1,45}$ = -2.44***	**0.45**	**$t_{1,43}$ = 3.20****	**0.44**	**$t_{1,43}$ = 0.3.14****
Root soluble sugars	-0.01	$t_{1,45}$ = -0.09	-	-	-	-
Root starch	0.05	$t_{1,45}$ = 0.33	-	-	-	-
Below ambient UV-B exposures						
Lupinus luteus						
Root flavonoids	**0.51**	**$t_{1,34}$ = 3.44****	0.22	$t_{1,34}$ = 1.29	-0.17	$t_{1,34}$ =-0.0107
Root anthocyanins	**0.36**	**$t_{1,34}$ = 2.19***	0.24	$t_{1,34}$ = 1.40	0.002	$t_{1,34}$ = 0.0313
Root soluble sugars	0.29	$t_{1,34}$ = 1.76	-	-	-	-
Root starch	0.15	$t_{1,34}$ = 0.89	-	-	-	-
Vicia atropurpurea						
Root flavonoids	-0.01	$t_{1,33}$ = -0.08	0.31	$t_{1,33}$ = 1.87	0.29	$t_{1,33}$ = 1.74
Root anthocyanins	-0.006	$t_{1,33}$ = -0.03	0.19	$t_{1,33}$ = 1.07	0.19	$t_{1,3341}$ = 1.08
Root soluble sugars	0.08	$t_{1,33}$ = 0.0.46	-	-	-	-
Root starch	0.31	$t_{1,33}$ = 1.86	-	-	-	-

To mimic field situation where N_2-fixing plants often depend on both soil-N and symbiotic-N for their N nutrition, 2 mM NO_3 was applied to nodulated plants of *L. luteus* and *V. atropurpurea*. Interestingly the extra N from NO_3 did not affect overall plant growth relative to purely symbiotic material, although leaf dry matter increased with reduced nodule %N in *L. luteus* (Tables 5.1 and 5.2). Supplying 2 mM NO_3 to *V. atropurpurea* also showed markedly ($P \leq 0.05$) increased nodule dry matter, but reduced root %N (Table 5.2). The overall lack of growth response by the two species to NO_3 supply is consistent with earlier reports by Ma *et al.* (1997) and Hill-Cottingham and Lloyd-Jones (1980).

Leaf concentrations of flavonoid-like compounds and anthocyanins decreased in *L. luteus*, just as root concentrations of flavonoid-like compounds, anthocyanins and soluble sugars were decreased with NO_3 supply to *V. atropurpurea* (Table 5.3). These changes in flavonoid-like compounds concentrations with N application have been observed previously (Cho & Harper 1991; Hunt & Mc Neil 1998; Khanna *et al.* 1999). Apparently, N fertilization of plants appears to have a general effect in down-regulating the phenylpropanoid pathway, usually leading to decreased tissue concentrations of most defence molecules such as phenols, phenol glucosides and condensed tannins (Bryant *et al. 1987*). It is possible that competition between C and N metabolism for ATP, NADPH as well as C skeletons needed in the synthesis of organic acids and carbohydrates on the one hand, and amino acids on the other, influences the shikimate pathway, thereby affecting the synthesis of phenylalanine-ammonia lyase (PAL). Being the enzyme that catalyses the first step of the phenylpropanoid pathway, a reduction in PAL would decrease tissue concentration of phenolic. But how NO_3 specifically inhibits the phenylpropanoid pathway, remains to be unravelled. In contrast, recent finding has also shown that nitrogen oxide (NO) formed during NO_3 reduction can increase radical formation with a potential to increase synthesis of phenolics/antioxidants under NO_3 fertilization (Stöhr and Ullrich 2002).

5.4.2 Below ambient UV-B effects on plant growth and symbiotic function

Plants of *L. luteus* and *V. atropurpurea* showed no changes in growth, symbiotic function and concentration of metabolites when cultured under below ambient UV-B, UV-A control or visible control (Tables 5.4, 5.5 and 5.6). This suggests that the

species involved are probably well adapted to growth in the presence of both UV-A and UV-B radiation. The lack of plant response to below ambient UV-B observed in this study agrees with reports by Tosserams *et al.* (1996) and Becwar *et al.* (1982). However, similar studies that attenuated or excluded UV-B and/or the UV-A component of natural solar radiation showed decreased biomass production in lettuce and cucumber cultivars (Krizek *et al.* 1998; Krizek *et al.* 1997). This is in contrast to reports by Pinto *et al.* (2002) and Shiokazi *et al.* (1999) where exposure of *P. vulgaris* and *P. sativum* to ambient UV-B radiation increased plant growth, nodulation and N_2-fixation. These inconsistencies in plant response to ambient levels of UV-B and/or UV-A could be attributed to genotypic differences.

However, feeding 2 mM NO_3 to *L. luteus* plants exposed to sub-ambient UV-B radiation markedly ($P \leq 0.05$) decreased total biomass and growth of individual organs, including root nodules (Table 5.4). Poor nodule development as a consequence of nitrate nutrition is a common feature of symbiotic legumes (Streeter 1988) which can lower nodule N concentration as observed in this study. Patterns of N allocation to organs were also altered with NO_3 supply as manifested by the increased concentration of N in leaves and stems of the two legumes relative to those of purely symbiotic plants (Table 5.5). Symbiotically speaking, rhizobial inoculation of legumes and nitrate feeding tend to produce directly opposite effects on tissue concentration of *nod* gene inducers. Whereas inoculating legumes with infective rhizobial cells increases the synthesis and release of *nod* gene inducers (Dakora *et al.* 1993) for increased nodule formation (Phillips *et al.* 1994), nitrate application decreases tissue levels of nod gene inducers (Cho and Harper, 1991) and thus limits the legume's nodulation potential. So relative to purely symbiotic plants, NO_3 supply to *L. luteus* probably decreased *nod* gene inducers with the consequent reduction in nodule formation, nodule biomass and total N content, as obtained here (Table 5.5). With *V. atropurpurea*, however, nodulation was not affected by NO_3 application (Table 5.5), possibly suggesting species differences in nitrate reduction of nod gene inducers in root tissues. This argument is supported by the fact that, with NO_3 supply, the concentrations of flavonoid-like compounds, anthocyanins, soluble sugars and starch were all significantly ($P \leq 0.05$) reduced in roots and to some extent leaves of *L. luteus*, but not in *V. atropurpurea* plants (Table 5.6). Although leaf anthocyanins were the only metabolites that decreased with NO_3

provision to *V. atropurpurea* plants grown in sub-ambient UV-B radiation (Table 5.6), being leaf-based, they were less likely to affect root nodulation. However, leaf and stem N concentrations were significantly ($P \leq 0.05$) increased in both legumes with NO_3 supply under sub-ambient UV-B conditions, suggesting changes in allocation patterns.

5.4.3 UV-B X N interactions

In this study, there were significant interactions between UV-B radiation and sources of N nutrition. When the effects of ambient and the two levels of elevated UV-B radiation were compared, it was apparent that in purely symbiotic *L. luteus* plants, total biomass and root anthocyanins were increased ($P \leq 0.05$) under UV-B_{173} compared to UV-B_{137} or ambient control (Tables 5.1 and 5.3). With NO_3-feeding, the leaf, nodule and total dry matter of *L. luteus*, as well as concentration of flavonoid-like compounds in leaves and plant total N were also markedly ($P \leq 0.05$) greater under UV-B_{173} relative to ambient or UV-B_{137} radiation (Tables 5.1, 5.2 and 5.3). From these interactions, it is probably fair to suggest that elevated UV-B, especially UV-B_{173}, results in increasing the accumulation of biomass, flavonoid-like compounds and anthocyanins in organs of NO_3-fed *L. luteus* plants. Chamber-grown *L. luteus* plants receiving sub-ambient UV-B and dependent on symbiosis for their N nutrition showed reduced leaf %N under UV-A compared to UV-B_{22} and visible control (Table 5.5). Their NO_3-fed counterparts also exhibited lowered root anthocyanins at UV-B_{22} compared to visible control (Table 5.6).

With *V. atropurpurea*, however, the UV-B X N interactions occurred mainly in the chamber-grown plants receiving sub-ambient UV-B radiation. Leaf and stem dry matter as well as root %N were increased ($P \leq 0.05$) in purely symbiotic *V. atropurpurea* plants grown under UV-B_{22} relative to those in visible control (Table 5.4). With NO_3 supply, stem dry matter together with root %N and root soluble sugars were markedly decreased ($P \leq 0.05$) under UV-B_{22} and UV-A compared to visible control (Tables 5.4, 5.5 and 5.6). So relative to UV-A and visible light, UV-B_{22} seems to increase biomass accumulation in leaves and stems, and %N in roots of purely symbiotic *V. atropurpurea* plants; however supplying NO_3 result in decreases of

these parameters. This suggests that NO_3 supply reduced resistance of *V. atropurpurea* plants to UV-B damage under the chamber conditions. Increased plant sensitivity to UV-B radiation with N application has also been observed in several studies (Correia *et al.* 2000; Hunt and McNeil 1998). Although the mechanisms for plant sensitivity to UV-B radiation with N application are not fully understood, it could be due to increased UV-B degradation of photosystem II proteins (Greenberg *et al.* 1989) which were reported to increase with N supply (Kolber *et al.* 1988). It has been reported that both UV-A and PAR have moderating effects on UV-B damage by inducing photo-reactivating processes that repair DNA lesions resulting from UV-B radiation (Jagger *et al.* 1969), and by stimulating biosynthesis of UV-B absorbing phenolics (Middleton and Teramura 1994). However, in UV-B attenuation studies, Krizek *et al.* (1998, 1997) showed that ambient UV-A reduced biomass production of *Lactuca sativa* (lettuce) and *Cucumis sativus* (cucumber) over and above that caused by ambient UV-B. It is therefore possible that the presence of UV-B and higher level of UV-A radiation in the UV-A control than the PAR control chamber (Figure 2.2B) adversely affected stem dry matter, root %N and root soluble sugars of the NO_3-fed *V. atropurpurea* plants.

This study also tested the effects of the two modes of N nutrition (purely symbiotic vs. symbiotic-plus-NO_3) on the plant's response to elevated UV-B radiation. A comparison at each level of elevated UV-B radiation revealed marked differences in species response. Relative to purely symbiotic plants, NO_3-fed *L. luteus* showed reduced ($P \leq 0.05$) nodule dry matter at UV-B_{100}, but greater concentration of flavonoid-like compounds in leaves and root anthocyanins at UV-B_{137} and UV-B_{173}, respectively (Appendices 2 and 3). In the chamber-grown sub-ambient UV-B plants, nodule mass and root anthocyanins of NO_3-fed *L. luteus* were also lower ($P \leq 0.05$) at UV-B_{22} relative to their purely symbiotic counterparts (Appendices 5 and 6). However leaf %N of NO_3-fed plants was greater ($P \leq 0.05$) than that of purely symbiotic plants under UV-A control (data not shown). With *V. atropurpurea*, however, nodule dry matter was increased ($P \leq 0.05$) in NO_3-fed compared to purely symbiotic plants grown under UV-B_{100} or UV-B_{137}. Plants grown in visible control showed greater ($P \leq 0.05$) root %N with NO_3 feeding compared to purely symbiotic counterparts. However plants receiving only UV-A radiation had less ($P \leq 0.05$)

soluble sugars in roots of NO_3-fed plants relative to those dependent on symbiotic N nutrition (Appendix 6). These interactive responses between elevated UV-B and NO_3 supply are consistent with data obtained by Musil and Wald (1994) which showed increased shoot biomass of *Dimorphotheca pluvialis* plants with higher UV-B radiation under high, but not low, nutrient conditions. However, our results contrast several reports on the increased sensitivity of plants to elevated UV-B radiation with high nutrient feeding (Correia *et al.* 2000; Hunt and McNeil 1998; Mural and Teramura 1985).

In conclusion, the results of this study show no adverse effect of elevated UV-B radiation (simulating 15 and 25% ozone depletion) on growth, and symbiotic function of *L. luteus* and *V. atropurpurea* plants. However, feeding NO_3 to nodulated plants of *L. luteus* under the highly elevated UV-B radiation promoted plant growth and total N content.

CHAPTER 6

RESPONSE TO ULTRAVIOLET-B RADIATION BY PURELY SYMBIOTIC AND NO_3-FED NODULATED TREE AND SHRUB LEGUMES INDIGENOUS TO SOUTHERN AFRICA

6.1 Introduction

Destruction of the ozone layer over the Antarctica and the Southern hemisphere (Crutzen 1992) interms of measured solar UV-B radiation is about 50% greater than that at comparable latitudes in the Northern hemisphere (Seckmeyer *et al.* 1995). Consequently, plants in the Southern hemisphere are exposed to more UV-B radiation than those in the Northern hemisphere. However, a large number of studies on the effects of UV-B radiation on plants have been conducted mostly in the Northern-hemisphere and centred on agricultural crop species with very few on trees in the Southern hemisphere (L'Hirondelle and Binder 2002). This is in-spite of trees accounting for up to 80% of global net primary productivity (Sullivan and Teramura 1989).

UV-B effects on plants include reduced biomass accumulation, altered biomass allocation to different plant organs and increased flavonoid content in plant tissues of sensitive species (Hofmann *et al.* 2001; Kolb *et al.* 2001; Lavola 1998; Schumaker *et al.* 1997). Growth response of tree species can be negative, beneficial or resistant to elevated UV-B (L'Hirondelle and Binder 2002; Laakso and Huttunen 1998). These differences in UV-B response may be due to plant genetic variation, influence of other stress factors such as drought or levels of mineral nutrients, the presence of protective features like waxy and reflective layers or thick epidermis, and UV-B accumulation of absorbing flavonoids (Bieza and Lois 2001, Correia *et al* 2000, Yuan *et al.* 2000, Jansen *et al.* 1998, Murali and Teramura 1987).

Although in the absence of tree harvesting, nutrient cycling in forests may not need N input beyond that which is non-symbiotic (Sprent 1987), there are many forest areas where N is limiting and nodulated legume trees form a major part of the flora (Sprent and Parsons 2000). Furthermore, biological nitrogen fixation is especially important in leguminous tree or shrub species that are used in Agroforestry (including shade and nurse crops) and land reclamation (Sprent and Parsons 2000). However, the few studies conducted on effects of elevated UV-B radiation on tree or shrub species paid little attention to their symbiotic function. For example, in studies with African *Acacia karroo* (Forsk.) Hayne, only nodule dry matter was assessed, and found to be unaltered with plant exposure to elevated UV-B radiation (Ernst et al. 1997, Wand *et*

al. 1996). Clearly, more studies are required to assess the effects of elevated UV-B radiation on tree and/or shrub legume nodulation and N_2 fixation. Because N-fed, but not N-depleted, maize (*Zea mays* L.) and cucumber (*Cucumis sativus* L.) plants were sensitive to elevated UV-B radiation (Correia *et al.* 2000; Hunt and McNeil 1998), we hypothesize that NO_3 additions in this study would increase plant sensitivity to UV-B radiation. The aim of this study was to determine the effects of UV-B radiation on growth, symbiotic function and concentration of metabolites of purely symbiotic and NO_3-fed nodulated tree and shrub legumes indigenous to Southern Africa.

6.2 Experimental

The legume plants under study included a commercially important herbal beverage in South Africa, *Cyclopia maculata* (L.) vent, and temperate evergreen species of *Podalyria calyptrata* Willd and *Virgilia oroboides* (Bergius T.M.) Salter. Seeds of the three test species were sown into potted sand, and germinated under different UV-B treatments. At emergence, seedlings of each species were inoculated with rhizobia isolates from nodules of the same species (section 2.1). Twelve separate tables with banks of fluorescent sun lamps (Philips TL/12 40W UV-B, The Netherlands) and 12 chambers constructed of differentially UV-transmitting clear perspex inter-dispersed in an open area were used (see section 2.2 for details). Each table or chamber had 4 pots with plants of each test species. For N treatment, 2 of the 4 pots received 1 mM KNO_3 while plants in the other 2 pots relied entirely on symbiotic N_2 fixation for their N nutrition. All pots were irrigated with equal volumes of water, and the seedlings later thinned out to two plants per pot. Immediately at emergence, 400 mL of ½ strength N-free Hoagland's nutrient solution (Hewitt 1966) was supplied twice weekly to the purely symbiotic plants and the same volume made up to 1 mM NO_3 applied to N-fed plants.

In this study, there were also two elevated UV-B treatments ($UV\text{-}B_{132}$ and $UV\text{-}B_{162}$) and one control ($UV\text{-}B_{100}$), each replicated four times. Measured UV-B exposures over the winter to summer growing period of plants averaged 98.3% (winter to summer range: 90.4% to 114.3%) of background in the $UV\text{-}B_{100}$ control, 142.0% (range 130.8% to 154.5%) of background in the $UV\text{-}B_{132}$ treatment and 171.5% (range: 139.4 to 193.4%) of background in the $UV\text{-}B_{162}$ treatment. Below ambient

UV-B treatments ($UV\text{-}B_{22}$), and two controls, one for photosynthetically active radiation (PAR control) and the other for UV-A (UV-A control) radiation, each replicated four times were also used (see section 2.2.for details). Average maximum daily air temperatures in the chambers (26.4 ± 0.57°C) and background (27.9 ± 0.61°C) were recorded for the growing period using temperature sensors.

Plants of *V. oroboides, P. calyprata* and *C. maculata* were harvested at 167, 184 and 194 d respectively after germination, and separated into nodules, roots, stems, leaves and pods where applicable. These plant organs were oven-dried at 60°C, weighed and ground into a fine powder for analysis of N and metabolites (section 2.2). Concentrations of N, flavonoid-like compounds and non-structural carbohydrates in all plant organs were measured as outlined in sections 2.4, 2.5, 2.6 respecticely and data analysed statistically using REML (residual maximum likelihood) variance component analysis (Genstat 1993) (section 2.8).

6.3 Results

Components of plant growth (leaf, stem, root, nodule or whole plant dry matter), symbiotic function (leaf, stem, root and nodule %N and total N content) and concentration of metabolites (flavonoid-like compounds, anthocyanins, soluble sugars and starch in the root, and flavonoid-like compounds and anthocyanins in the leaves) that were not significantly ($P \leq 0.05$) different with either UV-B exposure, NO_3 supply, or their interactions were not presented in the tables of the text, but were included in the appendices.

6.3.1 Above ambient UV-B effects

Plant growth

Exposing *C. maculata* to the highly elevated UV-B radiation reduced ($P \leq 0.05$) stem and leaf dry matter relative to the ambient UV-B; however root and total biomass were unchanged (Table 6.1). Total biomass and dry matter of individual organs of *V. oroboides* and *P. calyprata* were similarly unaltered with plant exposure to elevated UV-B radiation (Table 6.1). Supplying 1 mM NO_3 to *C. maculata* increased ($P \leq 0.001$) root dry matter but not stem, leaf or total biomass (Table1). The dry matter

yield of individual organs as well as plant total biomass of *V. oroboides* and *P. calyprata* also increased ($P \leq 0.001$) with NO_3 feeding (Table 6.1). There were significant interactions between elevated UV-B levels and N source on plant growth including root, stem and total dry matter in only *C. maculata* (Table 6.1). Mean separation of the effect of UV-B at each N source in this species showed that stem, root and total dry matter, of NO_3-fed plants were reduced ($P \leq 0.05$) by the moderately and/or highly elevated UV-B radiation (Figures 6.1A, B and D). In contrast, stem, root and total dry matter of purely symbiotic *C. maculata* were reduced ($P \leq 0.05$) by only the highly elevated UV-B radiation (Figures 6.1A, B and D).

Symbiotic performance

Components of symbiotic performance such as nodule dry matter, and plant N content were not affected in plants of *C. maculata*, *V. oroboides* and *P. calyprata* following exposure to elevated UV-B radiation (Table 6.1). However, leaf %N of *V. oroboides* decreased ($P \leq 0.05$) with plants growth under highly elevated UV-B (Table 6.1). Applying NO_3 to plants increased ($P \leq 0.01$) nodule dry matter of *V. oroboides* (Table 6.1). With *C. maculata*, however, only root %N was markedly ($P \leq 0.05$) increased with NO_3 feeding, in contrast to stem and leaf %N, which were significantly ($P \leq 0.05$) reduced (Table 6.1). Significant ($P \leq 0.05$) interactions between elevated UV-B and N source were apparent also in only *C. maculata* on components of symbiotic function such as nodule dry matter, nodule %N and total N content (Table 6.1). With NO_3-fed *C. maculata*, nodule dry matter, nodule %N and plant total were reduced ($P \leq 0.05$) with plant exposure to both moderately and highly elevated UV-B radiation (Figures 6.1C, E, and F), while their purely symbiotic counterparts, nodule %N and total N content were reduced in plants exposed to only the highly elevated UV-B (Figures 6.1C, E, and F). In fact, nodule dry matter of the purely symbiotic *C. maculata* was not altered with exposure to both moderately and highly elevated UV-B radiation (Figure 6.1C).

Table 6.1. Effects of elevated UV-B radiation on plant growth, symbiotic parameters and concentration of metabolites in purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within UV-B and N source treatments at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{134} = 34% above ambient ultraviolet-B, UV-B_{166} = 66% above ambient. DM = dry matter.
Measurements of components not listed under various parameters in this table were not significantly different, but were included in appendices 7 - 9.

Parameter	*Above ambient treatments*			N source		Wald χ^2 statistic		
	UV-B_{100}	UV-B_{134}	UV-B_{166}	Symbiotic-N	NO_3-N	UV-B (d.f. =2)	N (d.f. =1)	UV-B*N (d.f. = 2)
				Virgila oroboides				
Plant growth (DM, g plant^{-1})								
Leaf	9.85	9.11	8.63	**7.78b**	**10.62a**	0.83	**13.57*** **	1.46
Stem	8.13	7.57	6.65	**5.45b**	**9.45a**	2.11	**27.03*** **	2.45
Root	10.50	9.83	9.00	**8.00b**	**11.56a**	0.75	**19.62*** **	1.11
Nodule	1.88	1.80	1.73	**1.62b**	**1.99a**	0.78	**9.09****	0.50
Total plant	30.36	28.32	26.01	**22.84b**	**33.61a**	1.06	**22.64*** **	1.69
Symbiotic parameters								
Leaf %N	**2.55a**	**2.67a**	**2.25b**	2.39	2.59	**6.54***	2.69	2.59
Total plant N	635.08	598.27	509.60	**476.52b**	**685.44a**	2.34	**14.74*** **	1.60
Metabolites concentration								
Root soluble sugars (mg g^{-1})	20.99	25.44	25.16	**21.634b**	**26.09a**	2.21	**4.61***	0.63
Leaf anthocyanins (Abs g^{-1})	94.60	96.67	95.85	**91.235b**	**100.18a**	0.08	**7.01****	**10.12****
				Cyclopia maculata				
Plant growth (DM, g plant^{-1})								
Leaf	**10.28a**	**8.33ab**	**6.81b**	8.14	8.86	**5.93***	0.87	3.08
Stem	**7.09a**	**5.11ab**	**4.32b**	5.15	5.90	**8.10***	2.09	**7.69***
Root	11.08	9.06	9.14	**8.54b**	**10.95a**	1.25	**12.43*** **	**6.83****
Nodule	1.28	1.32	1.16	**1.38a**	**1.14b**	0.50	**5.19***	**6.84***
Total plant	29.73	23.81	21.43	23.20	26.85	4.84	3.42	**8.16***
Symbiotic parameters								
Nodule %N	4.14	3.73	3.536	**3.65b**	**3.96a**	5.18	**3.99***	**8.57***
Leaf %N	2.69	2.60	2.581	**2.88a**	**2.38b**	0.78	**16.61*** **	0.61
Total plant N	632.7	522.07	451.45	537.40	545.46	4.85	0.00	**8.68***
Metabolites concentration								
Root flavonoids (Abs g^{-1})	152.31	163.26	154.56	159.62	156.79	0.17	0.80	**11.42****
Root anthocyanins (Abs g^{-1})	1.38	1.529	1.33	**1.53a**	**1.30b**	2.01	**8.31***	0.90
Leaf anthocyanins (Abs g^{-1})	0.39	0.39	0.32	**0.42a**	**0.29b**	4.51	**6.65***	**6.64***
				Podalyria calyptrata				
Plant growth (DM, g plant^{-1})								
Leaf	10.96	9.54	10.67	**8.56b**	**12.23a**	1.09	**135*** **	0.17
Stem	6.16	5.59	6.06	**4.47b**	**7.401**	0.69	**18.45*** **	0.11
Root	10.84	10.76	10.30	**8.09b**	**13.17a**	0.33	**38.91*** **	0.86
Total plant	29.58	27.22	28.59	**22.58b**	**34.35a**	0.64	**21.06*** **	0.28
Symbiotic parameter								
Total plant N	552.6	506.92	542.65	**450.94b**	**612.94a**	0.51	**11.15*** **	0.24
Metabolites concentration								
Root soluble sugars (mg g^{-1})	17.95	15.34	19.50	17.22	17.97	3.48	0.00	**6.28***
Leaf flavonoids (Abs g^{-1})	179.05	179.49	182.64	**189.00a**	**171.75b**	0.08	**3.89***	3.75

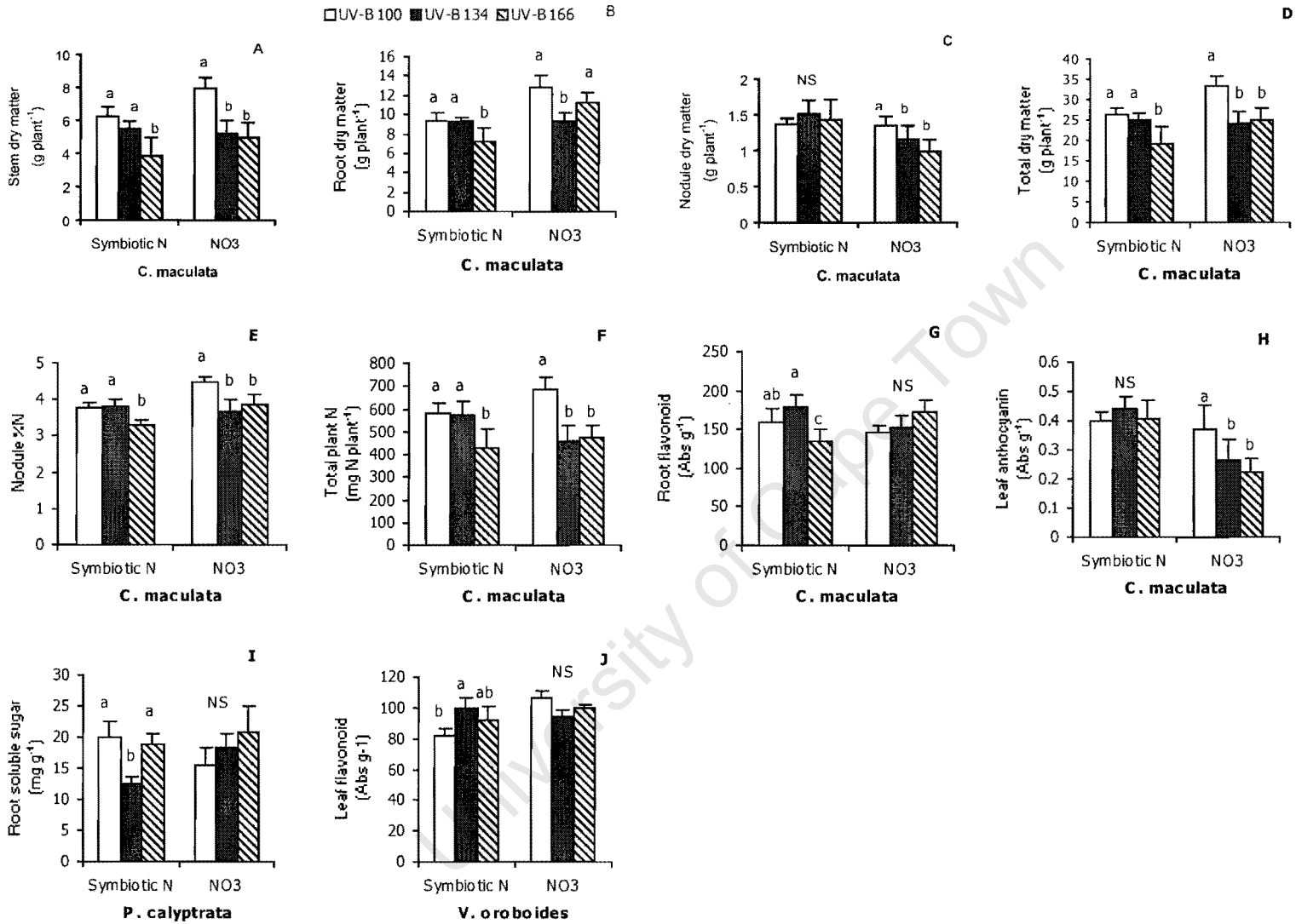

Figure 6.1. Interactive effects of elevated UV-B X N on plant growth, symbiotic function and concentration of metabolites in purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Vertical lines on bars represent standard error of mean. Dissimilar letters on bars within each N source indicate significantly different means at $P \leq 0.05$. NS = not significant, $UV\text{-}B_{100}$ = ambient ultraviolet-B radiation, $UV\text{-}B_{134}$ = 34% above ambient ultraviolet-B, $UV\text{-}B_{166}$ = 66% above ambient.

Tissue concentration of flavonoids and other metabolites

Exposing plants of *C. maculata*, *V. oroboides* and *P. calyprata* to elevated UV-B radiation did not alter the concentration of flavonoid-like compounds (A_b: 300 nm) and anthocyanins in roots and leaves, nor the levels of soluble sugars and starch in roots of any of the three species tested (Table 6.1) However, application of NO_3 to plants reduced ($P \leq 0.05$) the root and leaf concentration of anthocyanins in *C. maculata* (Table 6.1), and decreased leaf flavonoids in *P. calyptrata* (Table 6.1). This was in contrast to *V. oroboides,* which increased its concentration of flavonoid-like compounds in leaves and soluble sugars in roots with NO_3 supply (Table 6.1). Elevated UV-B radiation and N source interacted significantly ($P \leq 0.05$) on tissue phenolics such as root concentration of flavonoid-like compounds and leaf anthocyanins in *C. maculata* (Table 6.1), or leaf concentration of flavonoid-like compounds in *V. oroboides* (Table 6.1), and on soluble sugars in roots of *P. calyptrata* (Table 6.1). The moderately elevated UV-B radiation increased ($P \leq 0.05$) leaf concentration of flavonoid-like compounds in purely symbiotic plants of *V. oroboides* (Figure 6.1J), root concentration of flavonoid-like compounds in *C. maculata* (Figure 3G), but reduced root soluble sugars in those of *P. calyptrata* (Figure 6.1I). In NO_3-fed *C. maculata*, concentration of flavonoid-like compounds in roots was increased by the highly elevate UV-B (Figure 6.1G), but that of leaf anthocyanins was reduced by both the moderately and highly elevated UV-B radiation (Figure 6.1H).

6.3.2 Below ambient UV-B effects

Plant growth

Growing *C. maculata*, *V. oroboides* and *P. calyprata* plants in chambers under sub-ambient UV-B radiation did not alter organ growth or total biomass in any of the three species tested (Table 6.2). Application of 1 mM NO_3 to the plants increased stem, root and total dry matter of *V. oroboides* and/or *P. calyptrata* (Table 6.2). The interactions between sub-ambient UV-B radiation and N source on growth parameters were significant ($P \leq 0.05$) for dry matter of leaves and whole plants of *C. maculata* and *P. calyptrata*, and root and nodule dry matter for *C. maculata* and *P. calyptrata* respectively (Table 6.2). Separating the UV-B treatment effect on each N source showed that, relative to UV-A control but not PAR control, sub-ambient UV-

B_{22} exposure decreased ($P \leq 0.05$) leaf dry matter of purely symbiotic *C. maculata* and *P. calyptrata*, and total dry matter of the latter species (Figures 6.2A, F and I).

Interestingly, none of these growth parameters were altered in either purely symbiotic or NO_3-fed V. oroboides exposed to below ambient UV-B radiation (Appendix 10). However, symbiotically dependent plants of *C. maculata* in the chambers increased ($P \leq 0.05$) root and total dry matter with exposure to UV-A control relative to PAR control, but not UV-B_{22}, whereas their NO_3-fed counterparts were unchanged (Figures 6.2B and C).

Symbiotic performance

Exposing *C. maculata*, *V. oroboides* and *P. calyprata* plants to sub-ambient UV-B radiation had no effect on components of symbiotic function, including nodule dry matter and total N content (Table 6.2). However, leaf %N increased ($P \leq 0.01$) in *C. maculata* plants under UV-B_{22} relative to PAR control, but not to the UV-A control (Table 6.2). This was in contrast to nodule %N, which decreased ($P \leq 0.05$) in this species relative to UV-A control, but not to PAR control (Table 6.2). Stem %N in *P. calyptrata* also decreased ($P \leq 0.05$) under UV-B_{22} compared to UV-A control (Table 6.2). Feeding plants with NO_3 decreased nodule dry matter of *C. maculata* and *P. calyptrata* The %N in nodules and roots of *C. maculata* as well as total N of *V. oroboides* increased with NO_3 provision (Table 6.2).

The interactions between sub-ambient UV-B radiation and N source on symbiotic parameters were significant ($P \leq 0.05$) for root %N and total N of *V. oroboides* plants, total N content in *C. maculata* and nodule dry matter in *P. calyptrata* (Table 6.2). Symbiotically dependent *P. calyptrata* showed decreased nodule dry matter with UV-B_{22} exposure relative to UV-A control but not PAR control while their NO_3-fed counterparts remained unaltered (Figure 6.2G). Root %N of *V. oroboides* plants relying solely on symbiotic N increased ($P \leq 0.05$) with exposure to UV-B_{22} relative to PAR control but not UV-A control. This was in contrast to their NO_3-fed plants which showed reduced root %N relative to both UV-A and PAR controls (Figure 6.2I). Total plant N of symbiotically fed *C. maculata* increased with exposure to UV-A control

Table 6.2. Effects of below-ambient UV-B radiation on plant growth, symbiotic parameters and concentration of metabolites in purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within UV-B and N source treatments at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. $UV\text{-}B_{22}$ = 22% of ambient ultraviolet-B radiation, $UV\text{-}A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control. DM = dry matter.
Measurements of components not listed under various parameters in this table were not significantly different, but were included in appendices 10 – 12.

Parameter	Below ambient treatments			N source		Wald χ^2 statistic		
	$PAR_{cont.}$	$UV\text{-}A_{cont.}$	$UV\text{-}B_{22}$	Symbiotic-N	NO_3-N	UV-B (d.f. =2)	N (d.f. =1)	UV-B*N (d.f. = 2)
				Virgila oroboides				
Plant growth ***(DM, g plant^{-1})***								
Stem	5.41	5.29	4.55	**4.08b**	**6.09a**	1.22	**8.44****	3.92
Root	6.67	7.85	6.25	**6.10b**	**7.75a**	2.72	**7.12****	1.01
Total plant	18.42	19.28	16.21	**15.53b**	**20.41a**	1.90	**9.05****	4.59
Symbiotic parameters								
Root %N	1.60	1.75	1.60	1.61	1.69	2.71	2.22	**11.73****
Total plant N	407.46	442.50	344.43	**339.68b**	**456.68a**	2.97	**9.20****	**8.43***
Metabolites concentration								
Root flavonoids (Abs g^{-1})	61.31	52.93	50.59	**52.32b**	**57.56a**	2.10	**5.53***	**8.74***
Root soluble sugars (mg g^{-1})	13.80	13.49	15.04	**12.28b**	**15.94a**	0.14	**7.54****	1.28
				Cyclopia maculata				
Plant growth (DM, g plant^{-1})								
Leaf	4.48	5.01	3.96	4.47	4.50	1.15	0.31	**11.02***
Root	4.95	4.99	4.71	4.75	5.00	0.14	0.31	**5.92***
Nodule	0.46	0.50	0.47	**0.56a**	**0.39b**	0.40	**8.90****	0.09
Total plant	12.62	14.12	12.37	13.12	13.01	0.78	0.03	**6.45***
Symbiotic parameters								
Nodule %N	**4.27b**	**4.86a**	**4.28b**	**4.25b**	**4.70a**	**8.87***	**5.31***	0.88
Root %N	1.75	1.85	1.91	**1.76b**	**1.92a**	1.54	6.01*	3.82
Stem %N	**0.97b**	**1.12a**	**1.06ab**	1.09	1.02	**6.16***	3.33	1.35
Leaf %N	**2.23b**	**2.81a**	**2.63a**	2.84a	2.33b	**11.54****	**14.77*****	1.92
Total plant N	229.11	295.97	254.51	271.29	252.94	1.98	0.05	**6.60***
Metabolites concentration								
Root flavonoids (Abs g^{-1})	212.86	189.24	206.44	**220.00a**	**185.14b**	2.43	7.84*	**6.93***
Root anthocyanins (Abs g^{-1})	35.55	33.35	36.13	**37.01a**	**33.07b**	2.07	4.33*	0.43
				Podalyria calyptrata				
Plant growth (DM, g plant^{-1})								
Leaf	4.87	6.21	4.44	4.98	5.37	1.69	0.46	**6.81***
Root	5.71	7.18	5.71	**5.62b**	**6.78a**	2.40	**3.69**	4.12
Nodule	0.67	0.70	0.56	**0.74a**	**0.55b**	0.52	**6.07***	**6.77***
Total plant	13.53	17.47	12.94	14.00	15.30	1.95	0.89	**7.15***
Symbiotic parameter								
Stem %N	**1.19b**	**1.35a**	**1.19b**	1.25	1.23	**6.02***	0.07	2.14
Metabolites concentration								
Root anthocyanins (Abs g^{-1})	0.49	0.50	0.56	**0.57a**	**0.47b**	1.46	**5.37***	1.27
Root soluble sugars (mg g^{-1})	13.59	13.16	13.98	**15.79a**	**11.36b**	0.19	**8.84****	0.34
Leaf flavonoids (Abs g^{-1})	**119.35b**	**114.51b**	**139.19a**	123.48	123.14	**31.60*****	1.10	1.10

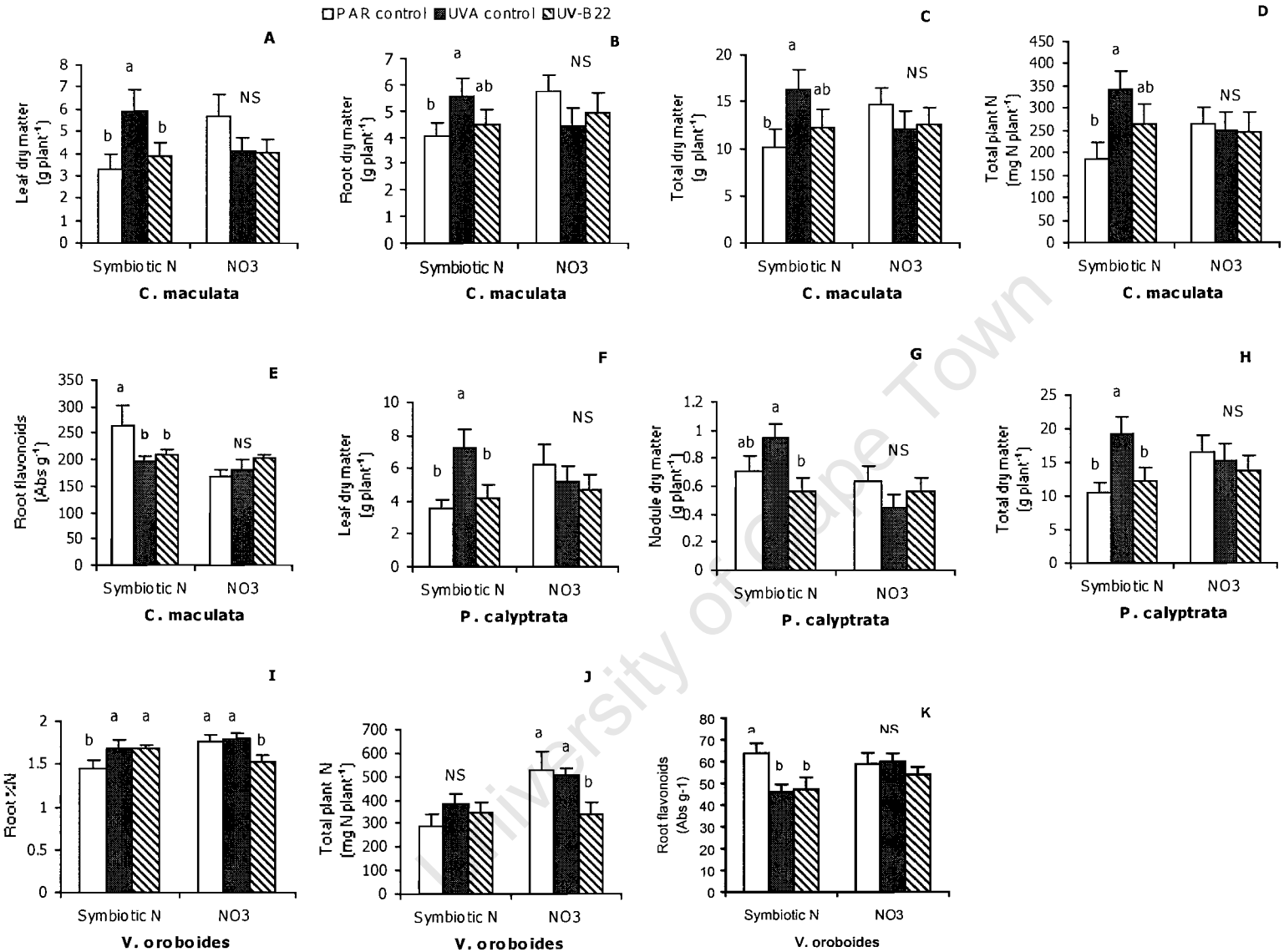

Figure 6.2. Interactive effects of below ambient UV-B X N on plant growth, symbiotic function and concentration of metabolites in purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Vertical lines on bars represent standard error of mean. Dissimilar letters on bars within each N source indicate significantly different means at $P \leq 0.05$. NS = not significant, UV-B_{22} = 22% of ambient ultraviolet-B radiation, UV-$A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control.

relative to PAR control but not UV-B_{22} (Figure 6.2D). This contrasted with *V. oroboides*, which showed reduced ($P \leq 0.05$) total plant N with exposure to UV-B_{22} relative to both UV-A and PAR controls, unlike their symbiotically dependent counterparts which remained unchanged (Figure 6.2J).

Tissue concentration of flavonoids and metabolites

The concentration of flavonoid-like compounds and anthocyanins in roots and leaves of the three test species were unchanged with sub-ambient UV-B radiation, except for flavonoid-like compounds in leaves of *P. calyptrata,* which increased ($P \leq 0.001$) under UV-B_{22} (Tables 6.2). Root concentrations of soluble sugars and starch were also not affected by exposure of the three species to sub-ambient UV-B radiation (Tables 6.2). However, NO_3 supply decreased root concentration of anthocyanins in *C. maculata* and *P. calyptrata* (Table 6.2). The concentration of flavonoid-like compounds in roots of *C. maculata* also decreased with NO_3 provision in contrast to *V. oroboides,* which increased considerably (Table 2). However, leaf concentrations of flavonoid-like compounds and anthocyanins were unaltered with NO_3 provision in all three species tested. The levels of soluble sugars increased ($P \leq 0.05$) with NO_3-application in roots of *V. oroboides*, but reduced in *P. calyptrata* (Tables 6.2). Significant ($P \leq 0.05$) interaction between sub-ambient UV-B radiation and N source was significant for only root concentration of flavonoid-like compounds in *C. maculata* and *V. oroboides* (Table 6.2). Relative to PAR control but not UV-A control, UV-B_{22} decreased root concentration of flavonoid-like compounds in both *C. maculata* and *V. oroboides* relying solely on symbiotic N, whereas their NO_3-fed counterparts were unaltered (Figures 6.2E and K).

6.4 Discussion

6.4.1 Above-ambient UV-B effects on plant growth and symbiotic function

Exposing plants to elevated UV-B radiation showed a marked decrease in stem and leaf dry matter of *C. maculata*, but not *P. calyptrata* or *V. oroboides* (Tables 6.1). At whole plant level, however, there were no major differences in response to elevated

UV-B. A number of studies (L'Hirondelle and Binder 2002; Laakso and Huttunen 1998) have shown that plants differ in their UV-B sensitivity and that species tolerance of UV-B may be genetically determined (Jansen *et al.* 1998). In general, tissue accumulation of UV-B absorbing flavonoids has been shown to be a common mechanism used by plants to reduce UV-B damage (Bieza and Lois 2001; Lavola 1998). The increased concentration of flavonoid-like compounds in leaves of *C. maculata* plants exposed to the highest UV-B radiation can therefore be interpreted as plant's attempt to overcome the damaging effects of UV-B radiation. The increase in tissue accumulation of flavonoid-like compounds not withstanding, *C maculata* was still more sensitive to UV-B compared to *V. oroboides* and *P. calyptrata*. This could suggest that, in this case, the greater tissue concentration of flavonoid-like compounds was not directly linked to defense against UV-B (Hunt and Kelliher 1996; Barnes *et al.* 1987), or that the levels achieved in C. *maculata* were still insufficient to avert UV-B damage. Although leaf and stem growth in *C. maculata* were altered with UV-B exposure, root and total dry matter was unchanged, suggesting altered biomass allocation with UV-B treatment as observed in other studies (Schumaker *et al.* 1997; Sullivan *et al.* 1994). The lack of growth response by *V. oroboides* (a tree) and *P. calyptrata* (a shrub) to elevated UV-B is consistent with data obtained for *Acacia karroo* (Ernst *et al.* 1997; Wand *et al.* 1996), a nodulating tree legume common to the African savanna.

As with plant growth, symbiotic performance was also not affected by exposure of *C. maculata*, *V. oroboides* and *P. calyptrata* plants to elevated UV-B radiation. For example, nodule dry matter, and total plant N were all unchanged with UV-B stress (Tables 6.1), an observation consistent with data obtained for other African tree legumes (Ernst *et al.* 1997; Wand *et al.* 1996). These findings however contrast the report by Singh (1997), which showed depressed symbiotic function in species of tropical grain legumes exposed to elevated UV-B. Although these inconsistencies in symbiotic response could be attributed to genotypic differences in UV-B sensitivity (Jansen *et al.* 1998), in this case it was more likely due to the high intensity of UV-B radiation used by Singh (1997), a daily dose of 10.28 kJ m^{-2} d^{-1} supplied over a 2-h period per day versus our daily dose of 4.50 or 8.05 kJ m^{-2} d^{-1} applied over an 8-h period per day to take into account the presence of ambient UV-B for almost the whole day.

Supplying nodulated plants with 1 mM NO_3 mimicked field situation where legumes often depend on soil-N and symbiotic-N for their N nutrition. The extra N provided as NO_3 to nodulated tree and shrub species under elevated UV-B in this study significantly ($P \leq 0.05$) increased overall growth by 47% in *V. oroboides*, 52% in *P. calyptrata* and by an insignificant 15% in C. *maculata* in comparison with purely symbiotic plants (Table 6.1). Although plant components such as leaves, stems and nodules contributed to total biomass, root growth was consistently much greater ($P \leq 0.05$) in all 3 species supplied with NO_3 relative to their purely symbiotic counterparts (Table 6.1). This is perhaps due to the signalling function of NO_3 (Crawford 1995) where exposure of plants to low concentrations (e.g. 1.0 mM) of this solute increased root biomass and lateral root development (Drew *et al.* 1973). Symbiotic response to NO_3 supply was different for the three legume species. Nodule development was, for example, depressed in *C. maculata* with NO_3 application, increased significantly ($P \leq 0.05$) in *V. oroboides*, but unaffected in *P. calyptrata*. In contrast, nodule activity was unifyingly enhanced ($P \leq 0.05$) by the supply of 1 mM NO_3 to all three species, a level of stimulation similar to that commonly obtained with starter-N application to legumes growing in low-N environments. While these results may be inconsistent with the findings of other studies (Ma *et al.* 1997; Hill-Cottingham & Lloyd-Jones 1980) where supplemental N had little effect on plant growth and symbiotic performance, they are in agreement with reports (Assefa and Ledin 2001; Del Pilar Cordovilla *et al.* 1999) which show that plant growth is increased with exogenous supply of N.

The increase in growth of *V. oroboides* and *P. calyptrata* plants with NO_3 supply was accompanied by a significant ($P \leq 0.05$) increase in total N content (Table 6.1). However, with *C. maculata*, where NO_3 had no effect on growth (Table 6.1), leaf and stem concentrations of N decreased ($P \leq 0.05$) with NO_3 feeding (Table 6.1). As with N, there was also a decrease in flavonoid-like compounds and anthocyanins of leaves and roots with NO_3 supply to *C. maculata* and *P. calyptrata* (Table 6.1). Such reductions in tissue concentration of flavonoid-like compounds with NO_3 provision has been observed before in soybean (Cho and Harper 1991) and cucumber (Hunt and McNeil 1998), and is apparently due to a generalised down-regulation of the phenylpropanoid pathway which results in decreased concentration of defence

molecules (Bryant *et al.* 1987). It is clear from these considerations that the decreased nodulation in *C. maculata* with NO_3 supply could be attributed to NO_3 inhibition of nodule formation (Streeter 1988) as a result of a lowered synthesis of *nod* gene inducers by roots (Cho & Harper 1991). Although the mechanism of NO_3 inhibition of the phenylpropanoid pathway by NO_3 remains unknown, it is possible that it is related to the reduction of C and N in cells. For example, if C and N metabolism compete for ATP, NADPH and C skeletons needed for synthesis of organic acids and carbohydrates on the one hand, and amino acids on the other, it will influence the shikimate pathway, therby affecting the formation of phenylalanine lyase (PAL). As the enzyme catalysing the first step of the phenylpropanoid pathway, a reduction in PAL synthesis will decrease tissue concentration of phenolics such as flavonoids and anthocyanins.

6.4.2 Below ambient UV-B effects on plant growth and symbiotic function

Culturing C. *maculata*, *V. oroboides* and *P. calyptrata* plants under below ambient UV-B radiation did not alter biomass accumulation, whether on whole plant or individual organ basis (Table 6.2). The lack of response to below-ambient UV-B radiation by *C. maculata*, which showed reduced leaf and stem growth under elevated UV-B, suggests growth adaptation at sub-ambient to ambient thresholds. These results are consistent with data obtained for UV-B exclusion and/or attenuation studies on trees and other species (Schumaker *et al.* 1997; Tosserams *et al.* 1996; Becwar *et al.* 1982), but contrast the increased biomass obtained when UV-B radiation was excluded from solar spectrum (Sharma *et al.* 1991; Bogenrieder and Klein 1982). However, promotion of growth, nodulation and N_2-fixation in *P. vulgaris* and *P. sativum* exposed to near ambient UV-B radiation have been reported (Pinto *et al* 2002; Shiokazi *et al.* 1999). These inconsistencies in plant growth response to below-ambient UV-B radiation could be purely genetic, or attributable to a number of factors such as the differences in materials used in UV-B attenuation studies, the period of plant exposure to UV-B especially when dealing with trees (Schumaker *et al.* 1997), or differences in ambient UV-B levels due to location of study (Tosserams *et al.* 1996).

Because plant growth was not altered by species exposure to below-ambient UV-B radiation, symbiotic performance was also not affected by sub-ambient UV-B (Table

6.2). However, relative to UV-A control but not PAR control, nodule %N of *C. maculata*, as well as stem %N of *P. calyptrata* plants were markedly reduced (Table 6.2). In contrast, %N in leaves of *C. maculata* plants under UV-B_{22} was significantly increased relative to PAR control but not UV-A control, suggesting that N uptake, translocation and/or storage in different plant organs were affected under the UV-B attenuation in the chambers. These changes can possibly be attributed to a number of factors in our experimental system such as the increased levels of UV-B and UV-A, the 0.8°C decrease in temperature between PAR and UV-A controls, and/or the altered spectral composition of light in the chambers (Figure 2.2B). However, because the air temperatures in UV-B_{22} and UV-A control were almost the same, and changes in %N of nodules in *C. maculata* and stems in *P. calyptrata* were different under UV-A control relative to both PAR control and UV-B_{22}, and because %N in stems and leaves of *C. maculata* were also different under UV-A relative to PAR control but not UV-B_{22}, (Table 6.2), changes in temperature and PAR could not have accounted for the observed differences. It is therefore more likely that the effects obtained were due to UV-A radiation. But the increase in leaf flavonoids of *P. calyptrata* plants exposed to UV-B_{22} relative to both UV-A and PAR controls, could be due to the effects of sub ambient UV-B radiation, a result consistent with other UV-B attenuation and/or exclusion studies (Searles *et al.* 1995; Lovelock *et al.* 1992).

Applying 1 mM NO_3 to all three test species under sub-ambient UV-B conditions increased ($P \leq 0.05$) root dry matter of *V. oroboides* and *P. calyptrata* plants. Furthermore, NO_3 supply to *V. oroboides* under sub-ambient radiation significantly ($P \leq 0.05$) increased whole-plant N (Table 6.2), which led to an increase in stem, root and total dry matter (Table 6.2) as well as an elevation in the concentration of flavonoid-like compounds and soluble sugars in roots of that species (Table 6.2). These increases with NO_3 supply show that nodule function in purely symbiotic plants was inadequate at meeting the different metabolic demands of plants exposed to elevated UV-B. However, the decrease in nodule dry matter of *P. calyptrata* fed with NO_3 suggests inhibition of nodulation by combined N (Del Pilar Cordovilla *et al.* 1999; Streeter 1988 and Senaratne *et al.* 1987). Root concentration of anthocyanins decreased ($P \leq 0.05$) with NO_3 feeding in both *C. maculata*, and *P. calyptrata*. Flavonoid-like compounds were similarly decreased in *C. maculata* but increased (P

≤ 0.05) in roots of *V. oroboides*. The reduction of flavonoid-like compounds and/or anthocyanins with NO_3 feeding is consistent with the down-regulation of the phenylpropanoid pathway (Bryant *et al.* 1983).

6.4.3 UV-B X N interactions

With exposure to supra-ambient UV-B conditions, stem, root and total dry matter, nodule %N and total plant N of purely symbiotic *C. maculata* were all depressed under UV-B_{166} relative to UV-B_{134} and ambient control. Nitrate application also resulted in a consistently decreased amount of dry matter in stems, roots, nodules and whole plants as well as a reduction in nodule %N and total plant N under the two levels of elevated UV-B relative to ambient control. Our data show that, although purely symbiotic *C. maculata* plants were sensitive to the higher UV-B radiation, adding NO_3 further increased their sensitivity to both levels of elevated UV-B, an observation consistent with the results of N and P supply to elevated UV-B plants (Correia *et al.* 2000, Hunt and McNeil 1998, Murali and Teramura 1985). Root concentration of flavonoid-like compounds in purely symbiotic plants were also low under UV-B_{166} relative UV-B_{134}, but increased ($P \leq 0.05$) with NO_3 feeding under UV-B_{166} relative to UV-B_{134} or ambient control. Whether this is due to PAL induction by NO from NO_3 (Stöhr and Ullrich 2002; Wendehenne *et al.* 2001), remains to be seen. In contrast, leaf anthocyanins were markedly decreased in UV-B_{166} relative to UV-B_{134}, which in turn was also more reduced than ambient control. In contrast to elevated UV-B conditions, NO_3 feeding under sub-ambient UV-B had no effect. However purely symbiotic *C. maculata* plants increased their leaf and total dry matter and total N under UV-A control relative to UV-B_{22} and PAR control. The leaf, nodule and total dry matter of *P. calyptrata* plants were similarly increased under UV-A relative to UV-B_{22} and PAR control. Root concentration of flavonoid-like compounds *in C. maculata* and *V. oroboides* were however decreased under UV-A and UV-B_{22} relative to PAR control.

When the two sources of N nutrition (purely symbiotic vs. symbiotic-plus-NO_3) were compared at each level of elevated and sub-ambient UV-B radiation, there was virtually no response by *V. oroboides* and *P calyptrata* at above-ambient conditions (Appendix 13-15). However, NO_3 feeding under PAR control increased ($P \leq 0.05$)

root %N and total N of *V. oroboides* relative to purely symbiotic plants, while NO_3 supply to *P. calyptrata* under UV-A decreased ($P \leq 0.05$) nodule dry matter compared to purely symbiotic material (Appendix 17). Unlike these two species, *C. maculata* plants showed differences in their response to N source at various levels of UV-B radiation. For example, relative to symbiotically-fed plants, those receiving supplemental NO_3 increased total dry matter under UV-B_{173} and nodule %N under UV-B_{100}, but decreased leaf anthocyanins under UV-B_{173} (Appendix 13-15). Although growth of C. *maculata* plants showed sensitivity to NO_3 application at the two levels of elevated UV-B radiation relative to ambient, N supply for growth appears limited under purely symbiotic fixation compared to NO_3 feeding. This is evidenced by the fact that dry matter yield of whole plants and individual organs were consistently higher under NO_3 nutrition than under purely symbiotic fixation. Similarly NO_3-fed plants of *C. maculata* increased total dry matter under UV-B_{22} and PAR control relative to purely symbiotic plants. But NO_3 nutrition decreased total dry matter under UV-A and root flavonoids under PAR control when compared to symbiosis-dependent N nutrition.

Because flavonoids are used to protect plant cells against UV-B damage (Bieza and Lois 2001; Mazza *et al.* 2000), their reduced concentrations in tissues from NO_3 feeding would be expected to increase sensitivity to UV-B (Sheahan 1996; Day *et al.* 1992). Although in this study, leaf concentration of flavonoid-like compounds in *P. calyptrata* plants decreased with NO_3 feeding (Table 6.1), a result consistent with reports by Khanna *et al.* (1999), Morandi and Le Qure (1991) and Cho and Harper (1991), the species was not adversely affected by elevated UV-B radiation. This suggests that other mechanisms are probably involved in plant sensitivity to UV-B radiation such as increased UV-B degradation of photosystem II proteins (Greenberg *et al.* 1989) which were reported to increase with N supply (Kolber *et al.* 1988).

In conclusion, exposing *V. oroboides* and *P. calyptrata* plants to elevated UV-B radiation did not affect their growth and symbiotic function. However feeding 1 mM NO_3 to these two species increased plant growth. But purely symbiotic plants of *C. maculata* were sensitive to the higher UV-B radiation, a response which increased with NO_3 supply.

CHAPTER 7

EFFECTS OF ELEVATED UV-B RADIATION ON PLANT GROWTH, N_2 FIXATION AND SEED YIELD OF *V. UNGUICULATA* AND *G. MAX* AND AN ASSESSMENT OF F1 GENERATION FOR CARRYOVER EFFECTS

7.1 Introduction

Reports of global ozone decline prompted research into the effects of increased solar UV-B on agricultural and natural ecosystems (Laakso and Huttunen, 1998). However, most studies examining UV-B effects on plants have been conducted on species of the Northern–hemisphere, and these have centered mostly on plant growth (biomass accumulation), physiological and morphological responses (Jansen *et al.* 1998). Some of those studies show that UV-B radiation can depress photosynthensis, reduce plant growth, induce photomorphogenic response, and enhance accumulation of defence compounds such as flavonoids (Mazza *et al.* 2000, Rozema *et al.* 1997; Teramura and Sullivan 1994). However information on UV-B effect on seed production is very limited, in the same way as data on UV-B carry over effects on offspring generation (Conner and Neumeier 2002).

Increases in UV-B radiation could potentially affect plant reproduction through reduction in number of flowers and seeds (Conner and Neumeier 2002; Sampson and Cane 1999) as a consequence of decreased photosynthesis and plant vegetative growth with UV-B exposure. Pollen germination and tube length can also be reduced by UV-B radiation in some species (Torabinejad *et al.* 1998; Musil 1995). In addition, UV-B can affect the ability of a plant to produce viable gametes or embryos by inducing deleterious mutations (Strid *et al.* 1994), and reduce pollination success by altering the ability of a plant to produce pollinator attractants (Conner and Zangori 1997). As a result, a decline in seed yield has been reported is some species exposed to elevated UV-B radiation (Mepsted *et al.* 1996; Teramura *et al.* 1990). In contrast, a number of reports have either shown no effect, or positive effects of elevated UV-B on plant growth and seed yield (Al-Oudat *et al.* 1997; van de Staaij *et al.* 1997; Musil 1995). For instance, increased seed production with UV-B exposure was reported in two species of *Brassica*, despite a known sensitivity to UV-B of other members of Brassicaceae (Wilson and Greenberg 1993).

Ultraviolet-B radiation effects on the parental generation can be carried over to offspring through mutation, maternal effects or epigenetic inheritance (Conner and Neumeier 2002; Musil 1996). This was evidenced in multi-generation studies where reductions in pigments, biomass and fecundity cumulated over multiple generations of

enhanced UV-B exposure and were seen as carryover effects of UV-B-induced damage to successive generations (Musil *et al.* 1999; Musil 1996). In contrast, Conner and Neumeier (2002) obtained increases in flower duration, total flower number, and percentage fruit set in parental plants exposed to UV-B, which was followed by increases in germination, plant height, leaf number, seeds per fruit, and total seed production in the F1 generation. These increases were attributed to UV-B-derived beneficial effects passed on by maternal plants to the offspring, or direct UV-B irradiance on seeds. It however remains to be seen whether UV-B effects on parental plant growth, symbiotic function and concentration of metabolites in *V. unguiculata* and *G. max* can be carried over to subsequent generations. This study assesses i) effects of elevated UV-B radiation on growth, N_2 fixation, concentration of metabolites and seed yield of *V. unguiculata* and *G. max*, and ii) UV-B-induced carry over effects in the F1 generation.

7.2 Experimental

7.2.1 Experiment 1

In Experiment one, plants of *Vigna unguiculata* (L.) Walp. (cowpea) landrace (Bengpilaa ex-Ghana) and *Glycine max* [L.] Merr (soybean) cv Prima were grown under elevated UV-B to seed maturity (see section 2.1 for details of plant culture). The experimental design comprised 12 separate tables with banks of fluorescent sun lamps (Philips TL/12 40W UV-B, The Netherlands) dispersed in an open area in the Kirstenbosch National Botanical Gardens, Cape Town. Twelve pots containing seeds of each test species were randomised on each table and germinated under UV-B radiation. There were two elevated UV-B treatments ($UV\text{-}B_{133}$ and $UV\text{-}B_{163}$) and one control ($UV\text{-}B_{100}$) each replicated four times. Measured UV-B exposures over the summer growing period of plants averaged 91.9% (range: 86.7% - 102.8%) of background in the $UV\text{-}B_{100}$ control, 132.2% (range 126.9% - 139.1%) of background in the $UV\text{-}B_{133}$ treatment and 158.5% (range: 153.6 - 166.2%) of background in the $UV\text{-}B_{163}$ treatment

Plants were harvested at physiological maturity at 135 d after germination for *G. max* and at 131 and 143 d after germination for *V. unguiculata*. The plants were separated into shoots, pods and nodulated roots, and roots and shoots oven-dried at 60°C to a

constant dry weight. Pods were also air-dried to a constant weight, shelled and the seeds per plant counted. After recording the dry weights, each sample was ground into a fine powder for analysis of N and metabolites.

7.2.2 Experiment 2

In Experiment two, seeds harvested from the UV-B exposed plants in Experiment one and original seed material were used. Plants were grown in a polycarbonate-clad greenhouse, which excludes any UV-B radiation. Two tables per species were used as a blocking factor with 48 pots of each test species randomly assigned to each table. Seeds from the parental plants exposed to elevated UV-B or ambient radiation and the original seed material were grouped into three seed size categories, except *G. max* original seed material which had only two seed size categories. The seed sizes for *G. max* measured in g seed^{-1}, were A) 0.147 (range: 0.140-0.154), B) 0.172 (range: 0.160-0.184) and C) 0.197 (range: 0.190-0.204). The seed sizes for *V. unguiculata* were A) 0.137 (range: 0.130-0.144), B) 0.157 (range: 0.150-0.164) and C) 0.177 (range: 0.170-0.184) g seed^{-1}. The seeds were grouped into different sizes to eliminate effects of seed size that might compound UV-B-induced carry over effects

Harvesting was done for only *G. max* during vegetative stage of growth at 65 and 68 d after emergence. *Vigna unguiculata* plants were discarded because their leaves were for some unknown reason chlorotic. The *G. max* plants were separated into stems, leaves, roots and nodules. The nodules were counted and all component organs oven-dried at 60°C to a constant dry weight. As with the other experiments, dry weights were recorded and each sample was ground into a fine powder for analysis of N and metabolites.

The concentration of N in plant organs from experiments one and two, as well as in seeds of the original material were measured (see section 2.4). Flavonoids, anthocyanins and non-structural carbohydrates were measured in both experiments following the procudure as outlined in sections 2.5 and 2.6.

A REML (residual maximum likelihood) variance component analysis (Genstat 1993) was used to statistically test treatment differences on each species (see section 2.8

for details). To determine the effect of previous parental exposure to elevated UV-B radiation on performance of F1 generation progenies and effect of seed size and their interaction, plants from seeds of the original material were excluded in the analysis because it had only 2 seed size categories (size A and B). Similarly, seed size C was excluded in the analysis when plants from seed of original material were included in order to compare performance between F1 generation progenies and original material. In either case, seed source and seed size (seed source*seed size) were inserted in the fixed model and plants per table in the random model.

7.3 Results

7.3.1 Experiment 1

UV-B effects on growth of parental plants

At physiological maturity, dry matter of *V. unguiculata* was not altered by both the moderately ($UV\text{-}B_{133}$) and highly elevated ($UV\text{-}B_{163}$) UV-B radiation whether on the basis of whole plant, nodulated root or shoot dry matter (Table 7.1). However, in *G. max*, the moderately elevated UV-B increased ($P \leq 0.05$) nodulated root dry matter while that of shoot or whole plant remained unchanged.

UV-B effects on symbiotic function of parental plants

Concentration of nitrogen in shoots of *V. unguiculata* increased ($P \leq 0.001$) with exposure to the highly elevated UV-B (Table 7.1). Nitrogen content in different organs (nodulated root, shoot, seed) or on whole plant basis as well as N fixed per plant were not altered with exposure to elevated UV-B. In *G. max* however, nitrogen content in shoot and whole plant vegetative biomass (i.e. excluding seed) increased ($P \leq 0.05$) with exposure to $UV\text{-}B_{163}$ relative to $UV\text{-}B_{133}$ but not ambient control (Table 7.1).

Concentration of metabolites in parental plants

Exposing *V. unguiculata* and *G. max* to elevated UV-B radiation did not alter concentration of metabolites such as flavonoid-like compounds (A_b: 300 nm),

anthocyanins, soluble sugars and starch in nodulated roots and shoots except shoot flavonoids of *G. max* which increased ($P \leq 0.05$) (Table 7.1).

UV-B effects on seed yield of parental plants

As shown in Table 7.1, pod number, pod dry matter, shelling percent, seed number, seed size and seed dry matter were not altered in both species except seed number in *G. max* which was reduced ($P \leq 0.05$) with exposure to elevated UV-B radiation.

7.3.2 Experiment 2

Carry over effects on soybean plant growth and symbiotic function

Comparing F1 generation progenies from parents previously exposed to elevated UV-B with ambient or original seed material did not show any change in growth whether on the basis of organ or total dry matter (Table 7.2). However, previous exposure to UV-B_{100} and/or UV-B_{133} reduced ($P \leq 0.05$) nodule %N and N content as well as whole plant N content and N fixed relative to plants from original seed material (Table 7.2). In contrast, concentration of leaf flavonoids increased ($P \leq 0.05$) with previous exposure to UV-B_{163} compared to UV-B_{100} or original seed material (Table 7.2).

When plants from original seed material were excluded in the analysis due to absence of seed size C, growth parameters of F1 generation progenies still remained unchanged with previous exposure to elevated UV-B (Table 7.3). However, increase in seed size significantly ($P \leq 0.001$) enhanced dry matter accumulation both on whole plant and individual organ basis (Table 7.3). There was no interaction between seed source and seed size on dry matter production of F1 generation *G. max* (Table 7.3). Previous exposure to elevated UV-B radiation of the parental plants also did not alter nodule number, nodule dry matter, total N content, total N fixed, N content and %N in all organs of the F1 generation progenies except in nodules where %N increased (Table 7.3). Similar to the growth parameters, nodule activity, organ or whole plant N content and N fixed were higher ($P \leq 0.05$ - 0.001) in larger seeded plants than their smaller seeded counterparts (Tables 7.3). With the symbiotic

Table 7.1. Effects of elevated UV-B radiation on growth, symbiotic function and seed yield of *Vigna unguiculata* and *Glycine max* plants. Significantly different means within treatments at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. $UV\text{-}B_{100}$ = ambient ultraviolet-B radiation, $UV\text{-}B_{133}$ = 33% above ambient ultraviolet-B, $UV\text{-}B_{163}$ = 63% above ambient. - = not determined, †Vegetative biomass excluding seeds.

	Vigna unguiculata				*Glycine max*			
	$UV\text{-}B_{100}$	$UV\text{-}B_{133}$	$UV\text{-}B_{163}$	Wald χ^2 statistics (d.f. = 2)	$UV\text{-}B_{100}$	$UV\text{-}B_{133}$	$UV\text{-}B_{163}$	Wald χ^2 statistics (d.f. = 2)
Roots (nodulated)								
Dry weight (g plant^{-1})	19.49	18.42	19.26	0.62	**19.23b**	**22.05a**	**19.27b**	**6.79***
N concentration (%)	2.19	1.95	2.06	2.42	2.76	2.73	2.61	1.59
N content (mg N plant^{-1})	427.92	369.03	337.84	1.57	510.13	575.77	462.82	3.17
Flavonoids (Abs g^{-1})	77.40	74.69	71.18	0.10	141.08	146.01	146.66	0.46
Anthocyanins (Abs g^{-1})	0.56	0.65	0.61	0.65	1.65	1.63	1.55	2.18
Soluble sugars (mg g^{-1})	48.57	47.16	40.91	3.00	18.86	19.60	20.46	0.12
Starch (mg g^{-1} DM)	90.43	92.90	96.06	1.26	93.87	94.14	92.75	0.10
Shoots								
Dry weight (g plant^{-1})	40.94	37.33	38.77	0.48	40.25	45.19	36.69	2.51
N concentration (%)	**1.83b**	**1.84b**	**2.31a**	**16.21*** **	2.04	2.13	2.05	0.91
N content (mg N plant^{-1})	740.59	728.02	865.44	4.20	**845.82ab**	**972.25b**	**729.16a**	**6.10***
Flavonoids (Abs g^{-1})	109.67	132.15	151.12	2.17	**182.02b**	**208.67a**	**207.90a**	**6.33***
Anthocyanins (Abs g^{-1})	0.87	1.03	1.71	2.63	1.214	1.24	1.18	0.14
Soluble sugars (mg g^{-1})	92.12	87.62	90.20	0.04	86.79	91.84	87.27	0.38
Starch (mg g^{-1} DM)	77.43	77.78	85.02	0.64	84.73	84.29	83.04	0.06
Grain yield								
Pod number plant-1	6.85	6.16	6.35	1.16	64.56	64.25	60.83	0.79
Total Pod DM (g plant-1)	14.21	14.55	13.11	2.05	30.09	29.07	30.00	1.35
Pod DM harvest 1 (g plant-1)	7.80	10.35	6.92	1.92	-	-	-	-
Pod DM harvest 2 (g plant-1)	6.40	4.20	6.19	1.71	-	-	-	-
Unseeded pod number plant-1	-	-	-	-	6.58	9.64	6.43	0.90
Unseeded pod DM (g plant-1)	-	-	-	-	0.66	1.06	0.61	1.41
Shelling percent	60.29	67.04	60.74	3.03	63.23	62.23	65.83	5.04
Seed number plant-1	58.11	62.18	55.15	1.61	**107.83a**	**99.94b**	**101.94b**	**9.23***
Seed dry matter (g plant-1)	8.66	9.74	8.30	3.93	18.74	17.50	19.28	3.71
Seed size (g seed^{-1})	0.15	0.16	0.15	3.09	0.172	0.18	0.19	3.82
N concentration in seed (%)	4.65	4.74	4.61	1.33	6.83	6.66	6.67	0.67
N content in seed (mg N plant^{-1})	395.14	460.55	377.06	4.56	1283.2	1165.0	1292.5	3.55
Whole plant								
Dry weight (g plant^{-1})	74.63	70.30	71.13	0.43	89.57a	96.31a	85.96a	2.96
†Vegetative mass N (mg plant^{-1})	1168.5	1097.1	1203.3	1.66	**1356.0ab**	**1548.0a**	**1192.0b**	**6.70***
N content (mg plant^{-1})	1582.7	1593.7	1581.9	0.03	2639.1	2713.0	2482.0	2.79
N fixed (mg plant^{-1})	1577.5	1588.5	1576.7	0.03	2630.0	2703.9	2472.8	2.78

parameters, interaction between seed source and seed size was significant ($P \leq 0.05$) for only %N in nodules (Table 7.2) where plant exposure to UV-B_{133} and/or UV-B_{163} increased ($P \leq 0.05$) nodule %N in the large seeded plants (Figure 7.1A).

Concentration of flavonoid-like compounds in roots and leaves of F1 generation *G. max* were not changed by previous exposure to elevated UV-B radiation (Table 7.3). Similarly, soluble sugars in the roots were unaltered, but starch levels increased with previous parental exposure to elevated UV-B radiation. Increases in seed size from 0.147g (size A) to 0.192 (size C) resulted in higher concentration of root flavonoid-like compounds, but root soluble sugars and starch were unaltered (Tables 7.3). Interaction between seed source and seed size were apparent for concentration of flavonoids in roots and leaves, and for root starch (Table 7.3). Concentration of flavonoid-like compounds in the roots increased with previous exposure to the highly elevated UV-B in size A, reduced in seed size B, but remained unchanged in seed size C (Figure 7.1B). Starch concentration in roots increased in seed sizes A and C, but remained unaltered in size B with previous exposure to elevated UV-B (Figure 7.1C). Similarly, concentration of flavonoid-like compounds in leaves of *G. max* were altered in seed size B and C but not in seed size A (Figure 7.1D).

7.4 Discussion

7.4.1 UV-B effects on growth, symbiotic function and seed yield of parental plants (Experiment 1)

Exposing parental plants to elevated UV-B radiation in this study did not alter total dry matter, symbiotic function or concentration of metabolites in roots and shoots of *V. unguiculata* and *G. max* at physiological maturity (Table 7.1). This is consistent with earlier data (Chapter 3) obtained for these species at flowering and pod formation stages. These findings are similar to those of Allen *et al.* (1999) and Stephen *et al.* (1999) which also showed unchanged biomass accumulation in legumes following exposure to elevated UV-B radiation. However, they contrast the results of Singh (1997, 1996), who found reduced plant biomass and decreased nodule numbers, nodule diameter and nitrogenase activity when closely related legume species such as (e.g. *Vigna radiata* and *Phaseolus mungo* were exposed to

Table 7.2. Carryover effects from previous exposure to elevated UV-B radiation on growth, symbiotic function and concentrations of metabolites of *G. max* F1 generation progenies. Significantly different means within treatments at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{133} = 33% above ambient ultraviolet-B, UV-B_{163} = 63% above ambient. Plants from category C of F1 seed material were excluded in the analysis for this table.

		Previous UV-B exposure			Wald χ^2 statistics
	Original material	UV-B_{100}	UV-B_{133}	UV-B_{163}	Seed source (d.f. = 3)
Nodules					
Nodule no.	36.61	42.25	31.92	41.36	6.42
Dry matter (mg plant^{-1})	0.22	0.21	0.19	0.20	2.42
N concentration (%)	**5.27a**	**5.08b**	**5.42a**	**5.65a**	**17.71*** **
N content (mg N plant^{-1})	**11.55a**	**10.40b**	**10.30b**	**11.27ab**	**8.73***
Activity (mg N fixed g^{-1} d^{-1})	5.47	5.43	5.08	5.69	3.74
Roots					
Dry matter (g plant^{-1})	0.51	0.53	0.47	0.58	3.75
N concentration (%)	2.23	2.06	2.00	1.98	2.01
N content (mg N plant^{-1})	11.45	10.78	8.97	10.53	6.54
Flavonoids (Abs g^{-1})	94.02	97.71	91.38	100.00	0.71
Soluble sugars (mg g^{-1})	25.54	22.56	19.14	24.57	2.73
Starch (mg g^{-1} DM)	80.24	86.28	88.54	93.62	5.20
Stems					
Dry matter (g plant^{-1})	0.46	0.46	0.43	0.459	1.09
N concentration (%)	4.20	4.24	4.18	4.19	
N content (mg N plant^{-1})	18.86	19.55	17.83	19.16	1.15
Leaves					
Dry matter (g plant^{-1})	1.28a	1.19	1.11	1.22	0.98
N concentration (%)	3.67a	3.67	3.21	3.65	3.85
N content (mg N plant^{-1})	45.02a	43.96	34.96	44.18	3.46
Flavonoids (Abs g^{-1})	**148.33b**	**156.53b**	**160.74ab**	**200.84a**	**10.02***
Whole plant					
Dry matter (mg plant^{-1})	2.47a	2.39	2.20	2.46	1.39
N content (mg plant^{-1})	**86.87a**	**84.68ab**	**72.07b**	**85.14ab**	**7.99***
N fixed (mg plant^{-1})	**77.73a**	**75.53ab**	**62.92b**	**75.99a**	**8.00***

Table 7.3. Carryover effects from previous exposure to elevated UV-B radiation on growth, symbiotic function and concentrations of metabolites of *G. max* F1 generation progenies. Significantly different means within treatments at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{133} = 33% above ambient ultraviolet-B, UV-B_{163} = 63% above ambient. - = not determined. Plants from the original seed material were excluded in the analysis for this table.

	Previous UV-B exposure			Seed size			Wald χ^2 statistics		
	UV-B_{100}	UV-B_{133}	UV-B_{163}	A (mean: 0.147g)	B (mean: 0.172g)	C (mean: 0.192g)	Seed source (d.f. = 2)	Seed size (d.f. = 2)	Source x size (d.f. = 4)
Nodules									
Nodule no.	42.79	35.73	44.12	37.71	41.06	42.89	5.58	2.79	2.00
Dry matter (mg plant^{-1})	0.22	0.21	0.20	**0.19b**	**0.21a**	**0.24a**	0.82	**15.09*** **	2.20
N concentration (%)	**5.13b**	**5.48a**	**5.60a**	5.40	5.28	5.43	**32.95*** **	2.45	**9.36***
N content (mg N plant^{-1})	11.03	11.69	11.35	**10.24b**	**10.89b**	**13.00a**	3.38	**14.85*** **	2.55
Activity (mg N fixed g^{-1} d^{-1})	5.56	5.37	6.04	**5.35b**	**5.43b**	**6.15a**	5.58	**7.88***	0.46
Roots									
Dry matter (g plant^{-1})	0.58	0.55	0.54	**0.46c**	**0.54b**	**0.69a**	3.78	**34.31*** **	5.28
N concentration (%)	2.01	2.03	2.15	2.01	2.11	2.04	0.50	0.01	7.99
N content (mg N plant^{-1})	11.50	11.03	11.45	**8.93c**	**11.21b**	**13.90a**	2.42	**43.25*** **	4.44
Flavonoids (Abs g^{-1})	102.46	91.48	99.52	**91.19b**	**99.21ab**	**103.03a**	3.03	**5.84***	**13.18***
Soluble sugars (mg g^{-1})	21.69	21.86	31.10	24.92	22.69	24.32	2.95	3.39	4.75
Starch (mg g^{-1} DM)	**79.80b**	**90.73a**	**99.86a**	90.19	90.29	85.56	**21.92*** **	1.20	**18.94*** **
Stems									
Dry matter (g plant^{-1})	0.50	0.49	0.47	**0.41c**	**0.49b**	**0.58a**	1.52	**28.23*** **	1.64
N concentration (%)	4.30	4.25	4.19	4.20	4.26	4.31	0.05	2.49	1.60
N content (mg N plant^{-1})	21.41	21.02	19.79	**17.13c**	**20.76b**	**24.94a**	1.24	**28.20*** **	0.58
Leaves									
Dry matter (g plant^{-1})	1.34	1.33	1.24	**1.05c**	**1.26b**	**1.64a**	0.61	**24.82*** **	0.73
N concentration (%)	3.48	3.25	3.65	**3.77a**	**3.27b**	**3.23a**	4.55	**7.56***	5.50
N content (mg N plant^{-1})	46.28	43.06	44.85	**40.40b**	**40.93ab**	**52.93a**	2.88	**11.70** **	0.73
Flavonoids (Abs g^{-1})	150.68	165.04	185.78	178.21	164.93	149.46	4.62	2.24	**12.42***
Whole plant									
Dry matter (mg plant^{-1})	2.64	2.59	2.45	**2.11c**	**2.50b**	**3.14a**	1.72	**31.57*** **	0.36
N content (mg plant^{-1})	90.25	86.80	87.43	**76.70b**	**83.82b**	**104.76a**	90.246	**20.43*** **	1.26
N fixed (mg plant^{-1})	81.10	77.66	78.28	**67.55b**	**74.67b**	**95.61a**	3.79	**20.05*** **	1.26

elevated UV-B radiation. As suggested previously, these inconsistencies in plant response to elevated UV-B radiation could be attributed to genotypic differences in sensitivity to UV-B (Jansen *et al.* 1998), the nutritional status of test plants (Correia *et al.* 2000), and/or the intensity of UV-B applied (Fiscus and Booker 1995).

The observed decrease in number of seeds per plant with the exposure of *G. max* to UV-B radiation (Table 7.1) is in agreement with results obtained for *Pisum sativum*, *G. max* and *P. mungo* (Mepsted *et al.* 1996; Singh 1995; Teramura *et al.* 1990). This reduction in seed number could be attributed to the UV-B effect on flower formation, pollination or, pollen germination and growth (Sampson and Cane 1999; Torabinejad *et al.* 1998; Strid *et al.* 1994; Flint and Caldwell 1984). Species differences were however apparent in this study as number of seeds per plant was reduced only in *G. max*, but not in *V. unguiculata* (Table 7.1). Although seed number per plant decreased in *G. max*, it was offset by an increase in seed size. Consequently, seed dry matter per plant was not altered with exposure to elevated UV-B radiation. Similar observations were reported by Mepsted *et al.* 1996 where significantly lower seed number per plant of *P. sativum* (cvs Montana and Orb) was followed by a higher, though not significant, average or total seed weight per plant with UV-B exposure. The increase in seed size with reduced number of seeds per plant can probably be explained by availability of more plant resources to the fewer seeds formed per plant than where the plant has more seeds (Carleton and Cooper 1972; Beveridge and Wilse 1959). The absence of seed yield response to elevated UV-B radiation observed in this study is in agreement with reports from other experiments involving legumes (Stephen *et al.* 1999; Al-Oudat *et al.* 1998), but contrasts with the findings of Mepsted *et al.* (1996); Singh (1995); Teramura *et al.* 1990). These variations in seed yield response to elevated UV-B radiation can also be attributed to genotypic differences in plant sensitivity to UV-B (Wilson and Greenberg 1993; Teramura *et al.* 1990), as well as different environmental conditions under which plants were grown, and/or the intensity of UV-B supplementation (Fiscus and Booker 1995).

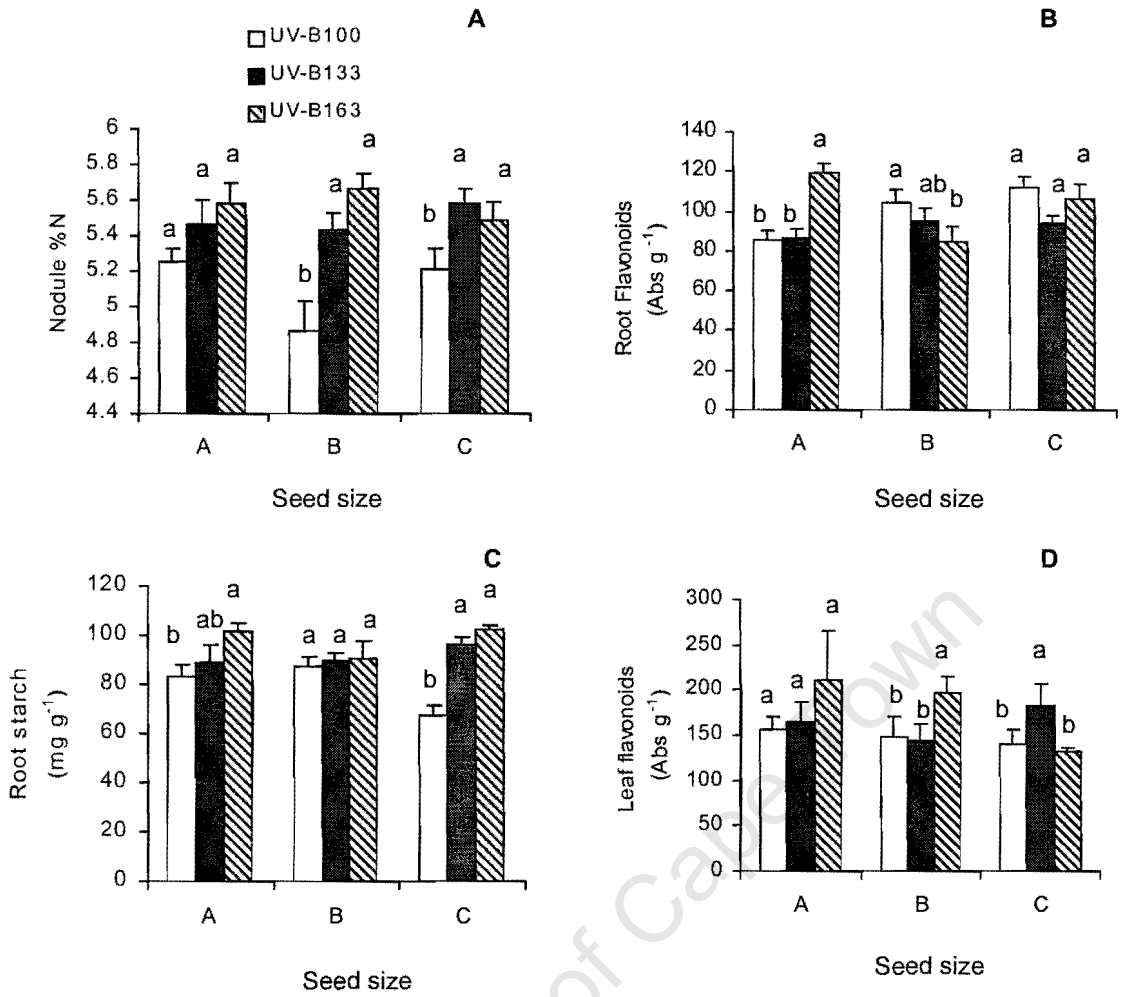

Figure 7.1 Interactive effects of previous exposure to UV-B X seed size on concentration of N and metabolites in different plant organs of F1 generation G. max. Vertical lines on bars represent standard error of mean. Dissimilar letters on bars within each seed size indicate significantly different means at $P \leq 0.05$. $UV\text{-}B_{100}$ = ambient ultraviolet-B radiation, $UV\text{-}B_{133}$ = 33% above ambient ultraviolet-B, $UV\text{-}B_{163}$ = 63% above ambient.

7.4.2 *UV-B induced carry over effects on plant growth and symbiotic function of F1 generation plants*

Relative to control (UV-B_{100}), nodule %N was significantly greater in F1 plants whose parents were exposed to elevated UV-B radiation. As a result, the total nodule N per plant was greater in progenies from the moderately elevated UV-B radiation (UV-B_{133}). However, these N values of F1 plants were not different from those of plants raised directly from the original parent material (Table 7.2). Although relative to UV-B_{100} control, F1 progenies from UV-B_{133} were lower in plant total-N and N fixed, the difference was not significant. Compared to plants from original seed, however, F1 plants from UV-B_{133} were significantly reduced in N fixed and therefore total plant N. Tissue concentration of flavonoids in F1 plants also showed a progressive increase with increase in UV-B radiation. In fact, there was 35% increase in concentration of flavonoid-like compounds in F1 plants from the highly elevated UV-B radiation relative to plants from the original parent seed and 28% compared to UV-B control (UV-B_{100}). These findings suggest that some subtle changes might have occurred in parental plants with exposure to UV-B, and these were then carried over to the subsequent F1 generation. The observed increase in concentration of flavonoid-like compounds in F1 generation plants from elevated UV-B would suggest a carryover effect on the phenylpropanoid pathway as an early alert for further defence against any rising UV-B radiation. In a similar study, Conner and Neumeier (2002) reported some beneficial effects of UV-B exposure on both the parents and F1 generation offspring of two wild species of *Phacelia*. They found an increase in flowering duration, total flower number, and percentage fruit set in the parental generation, as well as increase in seed germination, plant height, leaf number, seed number per fruit, and total seed production in the offspring.

A comparison of performance between the F1 generation progenies and original showed a decrease in total plant N, nodule N, and total N fixed in F1 generation plants whose parents were exposed to UV-B_{133} relative to plants from original seed material (Table 7.2). This negative effect on the F1 generation material exposed to the moderately elevated UV-B could be as a consequence of altered seed physiology and ultra-structure (Musil *et al.* 1998) or UV-B induced mutations (Strid *et al.* 1994). Furthermore, the multiple generation study on a desert annual *Dimorphotheca sinuata* has also shown the accumulation of negative effects of elevated UV-B radiation on

biomass and seed production as a consequence of altered DNA integrity (Musil *et al.* 1999; Musil 1996;).

7.4.3 Effects of seed size on plant growth and symbiotic function

Evaluating seed-size effects following UV-B exposure produced interesting results. Both organs and total dry matter increased with increasing seed size (Table 7.3) suggesting a positive correlation between seed size and seedling biomass. The results obtained here are consistent with those of other studies conducted on various plant species including: forage legumes (Carleton and Cooper 1972), *Medicago sativa* L. (Beveridge and Wilsie 1959), the clonal herb *Convallaria majalis* L. (Eriksson 1999), members of Australian and South African *Proteaceae* (Stock *et al.* 1990), and *Dactylis glomerata* L. (Bretagnolle *et al.* 1995). Although some reports have shown negative or no relationship between seed size and seedling growth (Humara *et al.* 2002; Gan and Stobbe 1996), the overwhelming evidence seem to suggest that large seeds generally produce higher seedling vigour and total biomass. This is because they possess larger embryos and larger quantities of cotyledonary reserves, which provide an advantage to seedling development especially under stress conditions such as low N (Cash and Ditterline 1996; Beveridge and Wilsie 1959). Black (1956) has however argued that the increase in plant growth of larger seeds than smaller seeds is related to the area of cotyledons rather than to their weight. This is supported by the fact that seed size correlated negatively with seed density (Sexton *et al.* 1997).

In their study, (Sexton *et al.* 1997) reported that *Phaseolus vulgaris* L. plants from large seeded background produced seeds which had greater cell volume, cell growth rate, seed growth rate and final seed size than smaller seeded plants. They further showed that the larger the seed cell volume, the greater the individual cell growth rate, and that volume per cell was the main determinant of final seed size. Bennett (1972) also observed a strong positive correlation between the quantity of nuclear DNA and cell length, or cell volume of herbaceous plants. This nucleotypic effect can have important impact on many morphological traits such as pollen diameter, size and weight of organs such as seed and leaves (Bennett 1972). Thus, increase in growth with large seeds can not be explained only by large seed reserves. It is therefore possible that the positive relationship between seed size and plant biomass of *G. max* in this study was due to greater seed reserves or large cell volumes with lots of

nuclear DNA which resulted in greater rate of cell growth and therefore bigger seedlings.

Because greater plant dry matter was observed for larger seeds, it was not surprising that nodule mass, nodule activity, organ N, total N and N fixed all increased with increasing seed size (Table 7.3), a result that is consistent with the findings of Cash and Ditterline (1996). Since N_2 fixation is highly dependent on photosynthates (Bethlenfalvay and Phillips, 1977) and positively correlates with plant dry matter, seed size if not properly accounted for, might confound estimates of N_2 fixation in legumes.

The finding that plant exposure to elevated UV-B radiation can reduce seed number per plant and that seed number correlates negatively with seed size in most species (Carleton and Cooper, 1972; Beveridge and Wilse 1959), such plants may produce fewer, but larger seeds due to high availability of photosynthates and plant reserves to the fewer number of flowers and/or pods (Shipley and Dion 1992; Venable 1992). Consequently, effects of elevated UV-B radiation could result in the production of larger but fewer number of UV-B sensitive plants at the ecosystem level.

In summary, data of this study show that seed yield of *V. unguiculata* and *G. max* was not adversely affected by plant exposure to elevated UV-B. However, although previous parental exposure to UV-B did not affect growth of F1 generation plants of *G. max*, the amount of N fixed per plant was reduced suggesting that subtle changes might have occurred in pathways of N metabolism with potential to accumulate in progenies with further exposures to UV-B radiation.

CHAPTER 8

USING ^{15}N NATURAL ABUNDANCE TO EXAMINE ULTRAVIOLET-B RADIATION EFFECT ON N METABOLISM IN SYMBIOTIC LEGUMES

8.1 Introduction

A number of studies have used ^{15}N natural abundance as tracer for N pools in the N economy of whole plants (Schulze *et al.* 1998; Pate *et al.* 1994; Kohl *et al.* 1980). This is based on the assumption that the $^{15}N/^{14}N$ isotopic ratio of N source is preserved during N absorption, assimilation and translocation. However, this assumption may not always hold because various physiological factors such as N uptake mechanisms, pathways of N assimilation, N recycling and N loss from plant can alter the $^{15}N/^{14}N$ ratio and discriminate against the heavier ^{15}N isotope (Wania *et al.* 2002; Evans 2001; Robinson *et al.* 1998). Environmental factors such as drought, N-starvation (Robinson *et al* 2000) and salinity (Handley *et al.* 1994) can also affect ^{15}N discrimination in tissues. In mutualistic associations involving plants and microbes, the isotopic fractionation of N is influenced by both the host plant and the rhizobial or fungal partner (Emmerton *et al.* 2001; Yoneyama *et al.* 2000; Steel *et al.* 1983;). Thus genotypic differences and/or intra-plant variations in N fractionation can seriously affect $\delta^{15}N$ values of organs (Handley and Scrimgeour 1997). The $\delta^{15}N$ value of a plant is therefore a function of its N source and the metabolic changes it has undergone with time. The $\delta^{15}N$ value of a plant can therefore be viewed as time- and mass-weighted average of N metabolism (Robinson *et al.* 1998) that integrates the plants' response to stress, and thus serve as a useful physiological integrator (Robinson *et al.* 2000).

A parallel to $\delta^{15}N$ is the use of $\delta^{13}C$ to measure a time-integrated value of internal CO_2 concentration and water use efficiency as factors affecting plant metabolism (Ormrod *et al.* 1997). Measurement of the stable carbon isotopic composition of a tissue can be a useful indicator of reduced growth response from photosynthetic damage by environmental factors. Ultraviolet-B radiation is an environmental factor of interest because of its photobiological effects in plants (Jansen *et al.* 1999). Ultraviolet-B radiation may penetrate plant tissue and directly damage cellular macromolecules such as proteins, nucleic acid enzymes, increasing oxygen free radical concentration and generally disrupting biochemical and physiological processes of plants (Teramura and Sullivan 1994). Adverse effects of UV-B radiation in plants include reduced plant growth, development and reproduction, and depressed photosynthesis (Tevini and Teramura 1989).

There are few studies that have examined UV-B radiation effects on carbon isotope composition, but with contradictory results. For example, Naidu *et al.* (1993) reported decreased $\delta^{13}C$ in *Pinus tadea* exposed to supplemental UV-B under field conditions, and attributed it to a reduced photosynthentic rate (Ormrods *et al.* 1997). Schmidt *et al.* (2000) also found lower $\delta^{13}C$ values in *Arabidopsis thaliana* under elevated UV-B, but attributed it to increased stomata conductance. In contrast, Kim *et al.* (1996) observed no significant changes in the proportion of $\delta^{13}C$ in tissues of three rice cultivars exposed to UV-B radiation. However, the effect of UV-B radiation on N isotope composition in plant tissues is lacking. The objective of this study was to determine the effect of UV-B radiation on $\delta^{15}N$ composition in legume plant organs and to determine whether $\delta^{15}N$ in organs correlates with the organ dry matter, %N and N content.

8.2 Experimental

In this study, plant samples collected from the eight legume species exposed to elevated UV-B radiation in experiments reported in Chapters 3, 5, 6, and 7 were analysed for $\delta^{15}N$ composition. The legume species include *Vigna unguiculata* (L.) Walp, *Glycine max* (L.) Merr, *Phaseolus vulgaris* (L.), *Lupinus luteus* (L.), *Vicia atropurpurea* (Desf.), *Virgilia oroboides* (Bergius T.M.) Salter, *Cyclopia maculata* (L.) and *Podalyria calyptrata* Willd. Seeds of these species were sown in potted sand, inoculated and grown under two levels of elevated UV-B radiation with a control (ambient) treatment (sections 2.1 and 2.2). However, material from F1 generation of *G. max* was collected from the experiment conducted in a polycarbonated-clad greenhouse which cuts off all ultraviolet radiation in order to assess UV-B induced carryover effects on $\delta^{15}N$ tissue composition.

Plants of *V. unguiculata, G. max* and *P. vulgaris* from experiment 1 were harvested 65, 68 or 72 d respectively after germination. At this time, *G. max* and *P. vulgaris* were at pod formation stage of growth, *V. unguiculata* was still at flowering stage. *Lupinus. luteus* was harvested at pod formation stage (108 d after germination), *V. atropurpurea* at flowering (128 d after germination) and plants of *C. genistoides, P. calyptrata* and *V. oroboides* were harvested at 167, 184, 194 d respectively, all in their vegetative stage of growth. Harvesting was done at flowering or early pod formation for the annual grain legumes (*V. unguiculata, G. max, P. vulgaris, L. luteus* and *V.*

atropurpurea) because it is the peak of nitrogen fixation, and for the tree and shrub legumes (*C. genistoides, P. calyptrata* and *V. oroboides*), a period of about 6 months UV-B exposure was considered adequate for assessment of UV-B effects. The ground powder from nodules, roots, stems, and leaves of plants that depended solely on symbiotic N was used for the determination of natural ^{15}N abundance.

Analysis of $\delta^{15}N$ in tissues

The ratio of $^{15}N/^{14}N$ measured as delta (δ) ^{15}N values (‰) in all plant organs and seeds of the parent material were analysed using a carlo Erba NA 1500 elemental analyzer (Fisons Insruments SpA, Strada Rivoltana, Italy) coupled to a Finnigan MAT 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) via a Conflo II open-split device. Whole-plant $\delta^{15}N$ value was calculated as an average of all plant organ $\delta^{15}N$ weighted by their respective total N contents (mg) (Robinson *et al.* 2000) as follows:

$$\text{Whole-plant } \delta^{15}N = \frac{(\text{leaf}\delta^{15}N \times \text{leafN}) + (\text{stem}\delta^{15}N \times \text{stemN}) + (\text{root}\delta^{15}N \times \text{rootN}) + (\text{nodule}\delta^{15}N \times \text{noduleN}) + (\text{pod}\delta^{15}N \times \text{podN})}{(\text{leafN} + \text{stemN} + \text{rootN} + \text{noduleN} + \text{podN})}$$

Similarly, shoot $\delta^{15}N$ was calculated as an average of leaf and stem $\delta^{15}N$ weighted by the total N contents of the leaf and stem, and nodulated root $\delta^{15}N$ was calculated as an average of root and nodule $\delta^{15}N$ weighted by their total N contents. Statistical analysis was performed on untransformed data using REML variance component analysis (Genstat 1993) (section 2.8) to test UV-B effects on tissue $\delta^{15}N$ composition. The analysis was done separately for each species and experiment.

8.3 Results

Effect of UV-B on $\delta^{15}N$ composition in plant organs

Exposing the legume plants to above ambient UV-B radiation did not affect $\delta^{15}N$ composition either on per organ basis or as whole plant in all the species except *C. maculata.* In this species, $\delta^{15}N$ in nodules became less positive, in contrast to the whole-plant $\delta^{15}N$ that was more positive with exposure to elevated UV-B radiation (Table 8.1). Similarly, during the assessment of carry over of UV-B effect to F1

Table 8.1. Effects of elevated UV-B radiation on N fractionation in different plant organs of five temoerate legumes. Significantly different means at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{132} = 32% above ambient ultraviolet-B, UV-B_{162} = 62% above ambient. - = not determined.

$\delta^{15}N$ of organ	Above ambient UV-B			Wald χ^2 Statistic
	UV-B_{100}	UV-B_{132}	UV-B_{162}	(d.f. = 2)
Vigna unguiculata				
Nodule	5.48	4.78	5.47	0.06
Root	-2.30	-1.14	-1.70	1.22
Stem	-0.70	-1.07	-0.36	2.24
Leaf	-	-	-	-
Whole plant	-1.06	2.71	1.95	1.71
Glycine max				
Nodule	6.09	6.00	6.23	1.11
Root	-	-	-	-
Stem	-0.95	-1.38	-0.97	2.27
Leaf	-1.49	-1.84	-1.56	2.26
Pod	-0.98	-1.07	-1.30	0.80
Whole plant	-1.12	-1.19	-1.13	0.09
Phaseolus vulgaris				
Nodule	9.10	9.84	9.72	1.36
Root	4.24	5.30	5.73	0.63
Stem	-3.62	-3.97	-3.41	1.09
Leaf	-2.59	-2.79	-2.04	2.74
Pod	-	-	-	-
Whole plant	0.42	1.02	0.04	1.53
Lupinus luteus				
Nodule	6.39	6.88	7.12	2.28
Root	-0.34	0.36	-0.15	0.99
Stem	-1.85	-1.97	-1.84	0.10
Leaf	-0.98	-1.00	-1.15	1.13
Pod	-0.43	-0.39	-0.61	0.78
Whole plant	0.54	0.32	0.31	0.73
Vicia atropupurea				
Nodule	0.01	0.001	0.20	0.78
Root	1.59	2.02	2.26	4.66
Stem	-1.50	-1.29	-1.19	4.02
Leaf	-0.29	-0.09	0.84	2.10
Whole plant	0.73	0.87	0.62	1.08
Cyclopia genistoides				
Nodule	**5.83a**	**5.29a**	**4.53b**	**12.03****
Root	-1.44	-1.52	-1.51	0.11
Stem	-2.91	-3.41	-3.40	0.47
Leaf	-1.29	-1.38	-1.09	1.26
Whole plant	**-0.14b**	**0.24ab**	**0.54a**	**6.26***
Virgilia oroboides				
Nodule	5.74	6.58	6.42	1.06
Root	-2.22	-1.76	-2.08	1.80
Stem	-2.18	-3.14	-3.41	1.92
Leaf	-2.06	-2.09	-2.24	0.80
Whole plant	0.02	0.04	0.05	0.01
Podalyria calytrata				
Nodule	3.67	3.70	3.71	0.01
Root	-1.59	-1.96	-1.73	2.05
Stem	-3.50	-3.06	-3.39	1.97
Leaf	-1.86	-1.49	-1.60	1.42
Whole plant	-0.12	0.17	0.15	1.97

generation progenies of *G. max*, $\delta^{15}N$ values of parental plants were not changed with exposure to above ambient UV-B radiation (Table 8.2). However, previous parental exposure to the highly elevated UV-B radiation significantly ($P < 0.001$) changed shoot and whole plant $\delta^{15}N$ of F1 generation plants relative to both plants from seeds from original material and from plants exposed to ambient UV-B control (Table 8.2). Shoot $\delta^{15}N$ became less negative where as that of whole plant was more positive (Table 8.2).

Correlations between $\delta^{15}N$ values and organ dry matter, %N and N content

Correlations between $\delta^{15}N$ values in organs and organ dry matter, %N and N content varied among organs and species (Tables 8.3 and 8.4). Values of $\delta^{15}N$ in nodules correlated negatively ($P < 0.05$) with %N in roots, leaves and stem of *V. unguiculata*, *G. max* and *P. vulgaris* respectively, and with stem dry matter and root N content in *V. atropurpurea* (Table 8.3 and 8.4). In contrast significantly ($P < 0.05$) positive correlations were observed between $\delta^{15}N$ in nodules and root, stem, leaf and total dry matter, %N in leaves, and nitrogen content in stems, leaves as well as total N content in *C. genistoides*, *P. calyptrata* and *V. oroboides*. Similarly, $\delta^{15}N$ in roots correlated negatively ($P < 0.05$) with nodule dry matter and nodule N content of both *L. luteus* and *C. genistoides*, but positively ($P < 0.05$) with leaf dry matter of *C. genistoides*, *P. calytrata* and *V. oroboides*; stem dry matter of *P. calyptrata* and *V. oroboides*; root and total dry matter, root %N, and root, leaf and total N content in *P. calyptrata* (Table 8.4). Correlations between $\delta^{15}N$ in stem and the plant parameters were apparent mostly in *G. max*, *P. vulgaris* and *V. oroboides* (Table 8.3 and 8.4). In these three species, stem $\delta^{15}N$ correlated positively ($P < 0.05$) with leaf dry matter, stem %N, stem and leaf N content. However, total dry matter and total N content correlated positively ($P < 0.05$) with stem $\delta^{15}N$ in only *G. max* and *P. calyptrata*.

There were also significantly ($P < 0.05$) positive correlations between leaf $\delta^{15}N$ and stem or root dry matter, stem N in *G. max*, *P. vulgaris*, and *V. oroboides* as well as with leaf %N in *V. atropurpurea* and *P. calyptrata*. Leaf $\delta^{15}N$ also correlated ($P < 0.05$) positively with total dry matter and total N in *G. max*, *leaf N* in *P. vulgaris*; and leaf %N in *V. atropurpurea* and *P. calyptrata*. Although leaf $\delta^{15}N$ correlated negatively with nodule %N in *L. luteus*, positive correlation was observed in *V. unguiculata* and

Table 8.2. Effects of elevated UV-B radiation on N fractionation in different plant organs of parental *G. max* and *V. unguiculata* plants and F1 generetion progenies of *G. max* grown without UV-B. Significantly different means at *P < 0.05, **P < 0.01, ***P < 0.001 in bold types and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{132} = 32% above ambient ultraviolet-B, UV-B_{162} = 62% above ambient, Prev. = previous UV-B exposure, - = not determined.

δ ^{15}N of organ	Parental plants (exposed to elevated UV-B)			Wald χ2 Statistic (d.f. = 2)	F1 generation progenies (grown without UV-B)				Wald χ2 Statistic (d.f. = 3)
	UV-B_{100}	UV-B_{133}	UV-B_{163}		Original material	Prev. UV-B_{100}	Prev. UV-B_{133}	Prev. UV-B_{163}	
Glycine max									
Seed	-0.56	-0.50	-0.60	0.21	-	-	-	-	-
Shoot	-1.95	-1.93	-1.95	0.02	**-1.05b**	**-1.02b**	**-0.89b**	**-0.65a**	**25.46*****
Root	2.72	2.38	2.38	1.51	2.80	2.84	2.83	2.81	0.32
Whole plant	-0.30	-0.27	-0.16	1.32	**0.57b**	**0.56b**	**0.66ab**	**0.76a**	**13.33****
Vigna unguiculata									
Seed	-1.47	-1.64	-1.47	1.60	-	-	-	-	-
Shoot	-2.01	-2.28	-1.67	3.20	-	-	-	-	-
Root	0.31	0.20	0.79	3.52	-	-	-	-	-
Whole plant	-1.14	-1.32	-0.87	3.54	-	-	-	-	-

Table 8.3: Correlations (Pearson product-moment coefficients, r) between $\delta^{15}N$ values in plant tissue and organ dry matter, %N and N content of tropical legumes exposed to above ambient UV-B radiation. Significant correlations at *P < 0.05, **P < 0.01, ***P < 0.001 in bold types.
Nodule, root, stem, and leaf dry matter, %N and N content not shown in a species have no significant correlation with any organ or whole-plant $\delta^{15}N$ values.

Species/Parameter	$\delta^{15}N$ of organ					
	nodules	root	stem	leaf	Shoot	Whole-plant
Vigna unguiculata						
Nodule %N	0.06	0.18	0.01	**-0.53***	-0.51*	-0.53*
Root %N	**-0.66****	**0.56***	-0.26	-0.04	-0.03	0-03
Leaf %N	-0.23	0.17	**0.46***	0.23	0.24	0.24
Glycine max						
Stem DM	-0.23	-0.09	0.39	**0.54***	0.49	0.10
Stem %N	-0.40	0.37	**0.63****	**0.52***	**0.66****	0.30
Stem N content	-3.7	0.11	**0.57***	**0.65****	**0.66****	0.20
Leaf DM	0.05	0.36	**0.72****	0.43	**0.65****	0.15
Leaf %N	**-0.74****	0.08	0.19	0.32	0.25	-0.25
Leaf N content	-0.15	0.37	**0.74****	0.49	**0.69****	0.08
Total DM	-0.15	0.09	**0.55***	**0.55***	**0.62***	0.323
Total N	-0.27	0.24	**0.66****	**0.66****	**0.71****	0.26
Phaseolus vulgaris						
Root DM	0.22	0.25	**0.59***	**0.53***	**0.62***	0.28
Root N content	0.20	0.17	**0.55***	0.46	**0.56***	0.27
Stem DM	0.28	0.24	0.47	**0.57***	**0.63****	0.15
Stem %N	**-0.55***	-0.17	**0.61***	0.16	-0.10	-0.28
Stem N content	0.22	0.27	**0.60***	**0.58***	**0.63****	0.14
Leaf DM	0.12	0.19	**0.58***	0.49	**0.59***	0.07
Leaf N content	0.13	0.21	**0.62***	**0.51***	**0.62***	0.06
Total DM	0.27	0.03	0.03	0.27	**0.54***	0.04
Total N	0.24	0.06	0.16	0.33	**0.60***	0.04

Table 8.4 Correlations (Pearson product-moment coefficients, r) between organ $\delta^{15}N$ values and organ dry matter, %N and N content of temperate legume exposed to above ambient UV-B radiation. Significant correlations at *P < 0.05, **P < 0.01, ***P < 0.001 in bold types.
Nodule, root, stem, and leaf dry matter, %N and N content not shown in a plant species have no significant correlation with any organ or whole-plant $\delta^{15}N$ values.

Species/Parameter	$\delta^{15}N$ of organs					
	Nodule	root	stem	leaf	Shoot	Whole-plant
Lupinus luteus						
Nodule DM	0.26	**-0.47***	0.02	-0.15	-0.18	-0.29
Nodule %N	0.01	0.02	**-0.60****	**-0.52***	0.11	-0.07
Nodule N content	0.27	**-0.44***	-0.07	-0.21	-0.14	-0.28
Root DM	0.18	-0.18	0.10	-0.07	-0.09	-0.37
Root %N	-0.05	**0.42***	0.04	0.22	0.05	0.15
Leaf DM	0.21	-0.15	0.03	0.03	-0.09	-0.33
Leaf N content	0.10	-0.12	0.07	0.07	-0.01	-0.30
Vicia atropurpurea						
Root %N	-0.04	0.29	-0.00	-0.02	0.09	0.21
Root N content	**-0.46***	0.06	-0.38	0.12	-0.12	-0.04
Stem DM	**-0.46***	0.23	**-0.53***	-0.13	0.03	0.12
Stem %N	0.30	0.19	**0.55***	0.03	0.43	0.35
Leaf %N	0.23	-0.12	0.26	**0.46***	0.11	0.24
Cyclopia genistoides						
Nodule DM	-0.26	**-0.63****	-0.04	0.29	-0.07	-0.16
Nodule N content	-0.22	**-0.59****	-0.03	0.32	-0.17	-0.24
Root DM	**0.43***	-0.35	0.03	0.24	-0.29	-0.23
Stem DM	**0.54****	-0.08	0.32	0.38	-0.22	-0.20
Stem N content	**0.57****	0.03	0.42	0.31	-0.21	-0.17
Leaf DM	**0.53***	-0.33	0.19	0.41	-0.36	-0.32
Leaf %N	**0.44***	**0.49***	0.19	-0.04	0.16	0.29
Leaf N content	**0.67****	-0.13	0.26	0.33	-0.32	-0.22
Total DM	**0.52***	-0.37	0.18	0.40	-0.33	-0.29
Total N	**0.76****	-0.21	0.26	0.36	-0.30	-0.24
Podalyria calytrata						
Nodule %N	**0.67*****	0.31	0.27	0.35	0.26	**0.54****
Root DM	**0.46***	**0.45***	0.25	0.32	0.23	0.40
Root %N	0.06	**0.56****	0.14	0.19	-0.05	-0.04
Root N content	0.38	**0.59****	0.27	0.31	0.18	0.28
Stem DM	**0.50***	**0.47***	0.21	0.26	0.30	**0.49***
Stem %N	**0.65****	0.23	0.17	0.19	0.22	**0.41***
Stem N content	**0.59****	0.51	0.24	0.28	0.33	**0.54****
Leaf DM	**0.54****	**0.51***	0.24	0.19	0.22	**0.42***
Leaf %N	0.36	0.78	0.10	**0.58****	0.21	0.30
Leaf N content	**0.58****	**0.52****	0.23	0.31	0.27	**0.51***
Total DM	**0.50***	**0.48***	0.23	0.25	0.29	**0.43***
Total N	**0.52****	**0.54****	0.25	0.30*	0.24	**0.44***
Virgilia oroboides						
Nodule %N	**0.91*****	**0.56****	0.28	**0.60****	0.02	0.27
Nodule N content	**0.55****	0.18	**0.43***	**0.51***	0.17	0.25
Root DM	**0.60****	0.19	0.28	**0.52****	0.14	0.16
Root N content	**0.61****	0.34	0.34	**0.57****	0.14	0.19
Stem DM	**0.60****	**0.48***	**0.43***	**0.61****	0.23	0.32
Stem %N	**0.56****	**0.44***	**0.55****	**0.71*****	0.01	0.07
Stem N content	**0.61****	**0.51***	**0.56****	**0.71*****	0.15	0.22
Leaf DM	**0.66****	**0.47***	**0.46***	0.30	0.30	0.33
Leaf %N	**0.64****	0.38	**0.52****	-0.02	-0.02	0.17
Leaf N content	**0.68*****	0.41	**0.59****	0.15	0.15	0.23
Total DM	**0.65****	0.40	**0.41***	0.24	0.24	0.28
Total N	**0.69*****	0.42*	**0.54****	0.16	0.16	0.24

V. oroboides. Shoot $\delta^{15}N$ correlated positively with leaf and total dry matter; stem, leaf and total N content in both *G max* and *P. vulgaris*, but with only stem %N in *G. max* and root dry matter, root N and stem dry matter in *P. vulgaris* (Table 8.3). Among all the legume species, whole-plant $\delta^{15}N$ in only *P. calyptrata* correlated positively with stem, leaf and total dry matter; nodule and stem %N; and stem and total N content (Table 8.4).

8.4 Discussion

The value of $\delta^{15}N$ in organs or whole-plants was not altered by exposure to above ambient UV-B radiation in the eight test species, except for *C. maculata*. Because the total $\delta^{15}N$ value of a plant is a function of the $\delta^{15}N$ values of the different external sources including fixation, as well as the $^{15}N/^{14}N$ fractionation associated with N uptake, transport, assimilation and excretion by the plant (Robinson *et al.* 2000), the lack of UV-B effect on $\delta^{15}N$ of these species could imply that none of these biochemical pathways was altered following UV-B exposure. However, the more positive whole-plant $\delta^{15}N$ values observed in *C. maculata* and the F1 generation of *G. max* plants suggest changes in some pathways of N metabolism when these species are exposed to UV-B radiation. In fact, various stress factors are known to alter $\delta^{15}N$ in plants. For example, Robinson *et al.* (2000) reported more negative $\delta^{15}N$ of whole-plants with imposition of drought or N starvation in *Hordeum spontaneum*, just as Handley *et al.* (1994) observed changed $\delta^{15}N$ values in the shoots of plants responding to salinity. The differences in $\delta^{15}N$ response by the eight test species to UV-B radiation are consistent with the results of $\delta^{13}C$ obtained for different plant species. For example, decreased $\delta^{13}C$ was reported for *G. max*, *Pinus tadea*, and *Arabidopsis thaliana* with exposure to UV-B (Feng *et al.* 2002; Schmidt *et al.* 2000; Ormrods *et al.* 1997; Naidu *et al.* 1993), while in three rice cultivars, the $\delta^{13}C$ was unaltered (Kim *et al.* 1996).

Robinson *et al.* (2000) have suggested that in experiments where exogenous N and its $\delta^{15}N$ values are known, whole-plant $\delta^{15}N$ may reflect the plant's net N retention because the general cause of $^{15}N/^{14}N$ discrimination between whole plant and

external N source is the loss from plants of some isotopically altered N. In this regard, they showed that genotypes that exhibited the largest ^{15}N discrimination (i.e. the most negative whole-plant $\delta^{15}N$ values) were the most productive and stress-tolerant and therefore retained the most N. Conversely, those genotypes that showed preference for 15N (i.e. most positive $\delta^{15}N$) were low in productivity, less tolerant to drought and N-starvation and contained low amounts of N. Similarly, in this study, *C. maculata* showed positive whole-plant $\delta^{15}N$ values with exposure to UV-B (Table 8.1) and contained significantly lower N content (Tables 6.2) when relying solely on symbiotic N and was therefore the most sensitive to elevated UV-B radiation. Interestingly, the F1 generation of *G. max* plants, which showed the more positive whole-plant $\delta^{15}N$ also recorded reduced amounts of N fixed per plant. This may suggest that there is a relationship between whole-plant or organ $\delta^{15}N$ and symbiotic function of plants. Additionally, the more positive whole-plant $\delta^{15}N$ coupled with the reduced amounts of N fixed in the F1 generation of *G. max* plants suggests subtle adverse UV-B effects had occurred in the pathway of N metabolism in parental plants exposed to elevated UV-B. Although these changes in N metabolism were undetected in parental plants, they were carried over to the F1 generation progenies, supporting the view that UV-B effects can accumulate over several generations (Musil et al. 1999).

Although the fractionation of $^{15}N/^{14}N$ was apparent in all the test species, it varied with species and organs (Table 8.1). For example, although there was ^{15}N enrichment in nodules of all test species except for *V. atropurpurea*, the extent of enrichment varied strongly, with the lowest nodule $\delta^{15}N$ being found in *P. calyptrata* ($\delta^{15}N$ = 3.67 ‰) and the highest in *P. vulgaris* ($\delta^{15}N$ = 9.10 ‰). Genotypic differences in ^{15}N discrimination have also been observed in studies involving *Hordeum vulgare* (Kolb and Evans 2003). The enrichment of ^{15}N in nodules could originate from both the host legume and the infecting rhizobial strain, from the accumulation of nitrogenous compounds such as γ-aminobutyric acid, the transport of ammonium out of bacteroids, the incorporation of amino acids and synthesis of various soluble N compounds inside nodules (Yoneyama 1988; Shearer *et al.* 1984; Freney and Gibson 1974). However, the leaf $\delta^{15}N$ values species were negative for both *V. atropurpurea* (-1.86 ‰) and *P. vulgaris* (–2.59 ‰) indicating isotopic

discrimination against ^{15}N during fixation of atmospheric N_2 by nitrogenase and transport proteins. This finding is in agreement with reports by Evans (2001), Yoneyama *et al.* 2001 and Kohl and Shearer (1980) where ^{15}N discrimination during N acquisition resulted in ^{15}N depletion of plants with respect to source. Although $^{15}N/^{14}N$ fractionation during N_2 fixation has been studied by a number of workers (Wanek and Arndt 2002; Tjepkema *et al.* 2000; Sprent *et al.* 1996; Yoneyama *et al.* 1986, Steele *et al.* 1983 and Shearer *et al.* 1982), the underlying isotope discrimination processes leading to non-uniform ^{15}N distribution within legumes remain poorly described (Wanek and Arndt 2002) despite developed models of the ^{15}N signatures for nitrate-fed plants (Robinson *et al.* 1998).

Although the correlations between $\delta^{15}N$ values and organ dry matter, %N and N content were species dependent, generally, the $\delta^{15}N$ in stem, leaf or shoot correlated positively with stem, leaf or total dry matter, as well as with stem %N and N content of stems and leaves or total N of *G. max* and *P. vulgaris* (Table 8.2). Also $\delta^{15}N$ of nodules and roots correlated positively with total dry matter, root, stem, or leaf dry matter, as well as with nodule, stem or leaf %N, and with stem, leaf or total N of *C. genistoides*, *P. calyptrata* and *V. oroboides* (Table 8.4). These correlations support the view that there is a link between $\delta^{15}N$ whether of organ or whole-plant and symbiotic parameters such as plant dry matter, %N and N content. With non-symbiotic systems, it might indicate the existence of a relationship bwtween whole-plant $\delta^{15}N$ and N metabolism in plants, eventhough Robinson *et al.* (2000) found no significant correlations between shoot $\delta^{15}N$ and shoot N or shoot %N, nor between root $\delta^{15}N$ and root N or root %N in *Hordeum spontaneum*. Because data is still insufficient in this area of study, especially between whole-plant $\delta^{15}N$ or organ $\delta^{15}N$ and physiological parameters, the use of $\delta^{15}N$ as a tool in understanding plant ecophysiology will require more studies to permit a better use of $\delta^{15}N$ as an integrator of physiological response to stress factors.

CHAPTER 9

GENERAL DISCUSSION

9 General discussion

The results of this study show that exposure to elevated UV-B radiation of food grain legumes such as *V. unguiculata*, *P. vulgaris* and *G. max* (Chapters 3 and 7); pasture legumes including *L. luteus* and *V. atropurpurea* (Chapter 5) and temperate evergreen species of *P. calyptrata* and *V. oroboides* (Chapter 6) did not alter plant growth, symbiotic function and seed yield. However, plant growth and N content of *C. maculata*, a commercially important herbal beverage in South Africa, were reduced with exposure to elevated UV-B (Chapter 6). These results have implications for agriculture, the environment and agroforestry. For instance, the unaltered seed yield of *V. unguiculata* and *G. max* with exposure to elevated UV-B (Chapter 7) suggests that production of food grain legumes is less likely to decrease with the anticipated increase in UV-B radiation due to stratospheric ozone depletion. Similarly, the unchanged biomass of pasture legumes indicates that livestock development and its commercial components such as meat, milk or wool production are unlikely to be adversely affected by elevated UV-B radiation as a consequence of decreased feed and forage production. The tolerance of *P. calyptrata* and *V. oroboides* to UV-B radiation also implies that their role in agroforestry systems is unlikely to be affected by rising UV-B radiation.

However, the decrease in biomass production by *C. maculata* with UV-B exposure could pose a threat to the development of the Honeybush tea industry in South Africa, as a decrease in leaf production would mean a drop in tea yield. This in return could reduce the export earnings derived from Honeybush tea for the South African economy. Because a lot of variations exist in the response of plant species or cultivars response to elevated UV-B radiation, there is a great chance of identifying *C. maculata* cultivars that are tolerant to elevated UV-B radiation. Thus, a selection program to select *C. maculata* cultivars that are tolerant of elevated UV-B radiation would be desirable.

The lack of response by plant growth and symbiotic performance in all the legume species tested (except *C. maculata*) indirectly suggests that UV-B did not adversely affect soil rhizobia *per se* possibly because UV-B radiation does not penetrate beyond 5 mm of the soil surface. Taken together, the data can be interpreted to

mean that with the lack of effect of UV-B on the legume host and rhizobial symbiont, the contribution of nitrogen fixation to soil fertility improvement may also not be adversely affected by increased UV-B radiation. However, where legume residue is constantly returned to the soil, this could lead to an increase in soil-N which enhances plant sensitivity to UV-B.

However, the response of purely symbiotic and NO_3-fed nodulated plants of *L. luteus*, *V. atropurpurea*, *C. maculata*, *P. calyptrata* and *V. oroboides* to UV-B radiation varied with species as growth and total N content of NO_3-fed plants increased in *L. luteus* but decreased in *C. maculata*. This suggests that the impact of the anticipated UV-B elevation on legume growth and symbiotic function could differ for agricultural and natural eco-systems depending on anthropogenic activities such as fertilizer application or atmospheric deposition. Furthermore, the increase or decrease in growth of some plants growing in N-rich environments with elevated UV-B radiation could alter species competitiveness leading to changes in vegetation and diversity at the ecosystem level.

Because exposure to elevated UV-B radiation can reduce seed number per plant (Chapter 7), and seed number correlates negatively with seed size in most species, such plants are likely to produce fewer but larger seeds due to high availability of photosynthate and plant reserves. Consequently, at the ecosystem level, the effects of elevated UV-B radiation on sensitive species could result in the production of fewer but larger plants, again altering the natural biodiversity. Additionally, because in this study the elevated UV-B radiation altered the concentration of nutrients in plants as well as tissue chemistry, the rate of litter decomposition could potentially increase or decrease depending on the plant species or organ. For most agricultural systems, decomposition is the key process driving nutrient release from soil organic matter thereby maintaining soil fertility. So UV-B-induced changes could significantly impact on nutrient cycling at the ecosystem level.

Overall, the results of this study also support the fact that some species are more tolerant than others to UV-B radiation. Of the eight test species only *C. maculata* was found to be sensitive to elevated UV-B radiation. It is possible that many more sensitive species exist. Therefore, further studies to examine a broader range of flora

is most desirable if we are to make sensible predictions about the overall ecological impacts of chronic stratospheric ozone depletions.

Although a very detailed and technically demanding study involving eight legume species was conducted, the results show that a rise in atmospheric UV-B radiation is unlikely to affect plant growth. However, because the study was descriptive rather than mechanistic, it is possible that subtle metabolic changes might have occurred but remain uncaptured by the techniques used. Consequently, without a more sensitive screen for UV-B responses, the likely impacts will remain hidden.

In conclusion, although several UV-B studies seem to give mixed signals on its impact on plant growth and metabolism, the data of this study show little or no evidence of UV-B-induced decrease in plant growth, symbiotic function and crop yield. Thus, the production of legumes whether for grain, pasture or agroforestry as well as the functioning of symbiotic microbial systems may not be affected with the increases in UV-B radiation.

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APPENDICES

Appendix 1. Effects of elevated UV-B radiation on organ or total dry matter accumulation of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea*. Significantly different means within N source treatment at $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ in bold type and separated by different letters. $UV\text{-}B_{100}$ = ambient ultraviolet-B radiation, $UV\text{-}B_{132}$ = 32% above ambient ultraviolet-B, $UV\text{-}B_{162}$ = 62% above ambient. - = not determined.

UV-B treatment	N Source	Plant growth (g plant^{-1}) Root dry matter	Stem dry matter	Leaf dry matter	Pod dry matter	Total dry matter
				Lupinus luteus		
$UV\text{-}B_{100}$	Symbiotic N	2.85	3.85	2.32a	0.43	9.93a
	NO_3-N	2.78	3.83	2.36a	0.26	9.58a
$UV\text{-}B_{132}$	Symbiotic N	2.69	3.16	2.08a	0.26	8.58a
	NO_3-N	3.13	3.88	2.46a	0.33	10.18a
$UV\text{-}B_{162}$	Symbiotic N	2.99	4.17	2.35a	0.44	10.38a
	NO_3-N	3.67	5.01	2.98a	0.43	12.59a
Wald χ^2 statistic						
UV-B (d.f.=2)		1.75	4.66	2.69	3.88	3.32
N (d.f.=1)		1.83	1.68	**4.72***	0.46	1.85
UV-BxN (d.f.=2)		4.29	3.42	**5.91***	1.96	**6.09***
				Vicia atropurpurea		
$UV\text{-}B_{100}$	Symbiotic N	7.93	5.33	5.80	-	19.22
	NO_3-N	6.21	4.35	6.17	-	17.04
$UV\text{-}B_{132}$	Symbiotic N	6.55	5.20	5.35	-	17.23
	NO_3-N	6.23	4.92	6.39	-	17.88
$UV\text{-}B_{162}$	Symbiotic N	6.30	4.50	5.74	-	16.77
	NO_3-N	5.73	4.54	5.26	-	15.78
Wald χ^2 statistic						
UV-B (d.f.=2)		1.40	0.54	0.57	-	0.77
N (d.f.=1)		1.88	0.69	0.33	-	0.54
UV-BxN (d.f.=2)		0.35	0.63	0.92	-	0.51

Appendix 2. Effects of elevated UV-B radiation on symbiotic parameters of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea*. Significantly different means within N source treatment at $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{132} = 32% above ambient ultraviolet-B, UV-B_{162} = 62% above ambient. - = not determined.

UV-B treatment	N source	Nodule dry matter (g plant^{-1})	Nodule activity (mg N fixed g^{-1}d^{-1})	Root %N	Stem %N	Leaf %N	Pod %N	Total N content (mg plant^{-1})
				Lupinus luteus				
UV-B_{100}	Symbiotic N	**0.49a**	6.24	1.38	2.15	5.52	5.29	309.9a
	NO_3-N	**0.35b**	7.95	1.26	2.27	5.40	4.96	286.3a
UV-B_{132}	Symbiotic N	0.40a	8.42	1.56	2.56	5.77	5.22	287.1a
	NO_3-N	0.38a	7.83	1.47	2.14	5.45	4.71	305.9a
UV-B_{162}	Symbiotic N	0.44a	7.26	1.69	2.28	5.14	4.87	318.6a
	NO_3-N	0.50a	7.39	1.50	2.04	5.33	5.16	372.2a
Wald χ^2 statistic								
UV-B (d.f.=2)		1.65	1.20	3.63	4.71	**6.26***	0.70	3.03
N (d.f.=1)		1.52	2.97	1.63	3.51	0.35	0.40	0.24
UV-BxN (d.f.=2)		**11.24****	3.18	0.42	5.42	2.44	2.73	**6.98***
				Vicia atropurpurea				
UV-B_{100}	Symbiotic N	**0.16b**	**32.22a**	2.55	2.36	3.83	-	542.6
	NO_3-N	**0.31a**	**14.84b**	2.42	2.52	3.78	-	506.4
UV-B_{132}	Symbiotic N	**0.13b**	**40.06a**	2.87	2.48	3.84	-	522.4
	NO_3-N	**0.34a**	**13.33b**	2.25	2.48	4.15	-	539.6
UV-B_{162}	Symbiotic N	0.23a	21.14a	2.93	2.56	3.93	-	531.5
	NO_3-N	0.25a	15.55a	2.30	2.30	4.02	-	459.5
Wald χ^2 statistic								
UV-B (d.f.=2)		0.30	0.28	0.50	0.31	1.73	-	0.30
N (d.f.=1)		**9.04****	**13.03*****	**6.79***	0.14	0.60	-	0.94
UV-BxN (d.f.=2)		**6.53***	**6.12***	1.28	3.29	0.68	-	0.37

Appendix 3. Effects of elevated UV-B radiation on concentration of flavonoids, anthocyanins and non-structural carbohydrates in roots or leaves of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea*. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{132} = 32% above ambient ultraviolet-B, UV-B_{162} = 62% above ambient.

UV-B Treatment	N source	Root flavanoids (Abs g^{-1})	Root anthocyanins (Abs g^{-1} DM)	Root soluble sugars (mg g^{-1} DM)	Root starch (mg g^{-1} DM)	leaf flavanoids (Abs g^{-1} DM)	Leaf anthocyanins (Abs g^{-1} DM)
				Lupinus luteus			
UV-B_{100}	Symbiotic N	107.2	0.65a	4.43	72.44	204.1a	1.29
	NO_3-N	89.2	0.53a	3.52	73.66	189.7a	1.05
UV-B_{132}	Symbiotic N	111.0	0.67a	**4.20**	**66.96**	**210.0a**	**1.35**
	NO_3-N	91.5	0.70a	2.52	76.50	**175.7b**	0.68
UV-B_{162}	Symbiotic N	152.2	**1.15a**	5.41	73.63	205.2a	1.74
	NO_3-N	144.7	**0.78b**	2.59	79.11	207.9a	1.66
Wald χ^2 statistic							
UV-B (d.f.=2)		**9.03***	1.05	0.70	0.15	2.26	**6.71***
N (d.f.=1)		1.97	2.22	2.23	1.67	**3.74***	**4.84***
UV-BxN (d.f.=2)		0.99	**6.15***	1.43	0.33	**5.93***	4.07
				Vicia atropurpurea			
UV-B_{100}	Symbiotic N	68.71	1.42	7.92	60.45	141.5	4.26
	NO_3-N	53.35	0.77	7.39	57.95	123.0	1.43
UV-B_{132}	Symbiotic N	63.87	1.18	8.21	58.44	137.5	4.99
	NO_3-N	52.23	0.86	6.65	61.40	138.7	2.00
UV-B_{162}	Symbiotic N	69.77	1.48	10.28	67.52	139.9	3.02
	NO_3-N	40.25	0.81	6.46	62.38	109.4	1.17
Wald χ^2 statistic							
UV-B (d.f.=2)		0.32	1.49	0.59	1.01	3.30	2.91
N (d.f.=1)		**19.98*****	**42.08*****	**6.73***	0.22	**6.42***	**38.15*****
UV-BxN (d.f.=2)		2.57	1.24	2.76	0.93	4.65	0.17

Appendix 4. Effects of below-ambient UV-B radiation on organ or total dry matter accumulation of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea*. Significantly different means within N source treatment at $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{132} = 32% above ambient ultraviolet-B, UV-B_{162} = 62% above ambient. - = not determined.

UV-B treatment	N source	Plant growth (g plant^{-1}) Root dry matter	Stem dry matter	Leaf dry matter	Pod dry matter	Total dry matter
			Lupinus luteus			
$Visible_{cont.}$	Symbiotic N	2.23a	3.57	2.47	1.51	10.19
	NO_3-N	2.40a	3.07	2.37	1.07	9.23
UV-$A_{cont.}$	Symbiotic N	2.75a	4.74	3.52	2.05	13.61
	NO_3-N	2.66a	3.12	2.33	0.68	9.09
UV-B_{22}	Symbiotic N	**2.71a**	4.05	3.16	1.45	11.84
	NO_3-N	**2.31b**	3.02	2.51	1.04	9.21
Wald χ^2 statistic						
UV-B (d.f.=2)		0.19	0.33	0.06	0.87	0.31
N (d.f.=1)		1.45	**10.42****	**4.95***	**4.58***	**13.47*****
UV-BxN (d.f.=2)		**6.75***	5.11	5.19	2.68	5.53
			Vicia atropurpurea			
$Visible_{cont.}$	Symbiotic N	3.88	5.01a	5.57a	-	14.64a
	NO_3-N	2.40	4.20a	4.52a	-	11.75a
UV-$A_{cont.}$	Symbiotic N	3.34	4.65a	4.88b	-	13.09a
	NO_3-N	2.04	2.77a	2.80a	-	7.85a
UV-B_{22}	Symbiotic N	2.64	8.16a	8.38a	-	19.46a
	NO_3-N	1.62	2.11a	2.62a	-	6.48a
Wald χ^2 statistic						
UV-B (d.f.=2)		1.04	0.07	0.24	-	0.07
N (d.f.=1)		**6.20***	**7.04****	**4.06***	-	**7.54****
UV-BxN (d.f.=2)		0.12	**13.06****	**8.84***	-	**9.04***

Appendix 5. Effects of below-ambient UV-B radiation on symbiotic parameters of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea*. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{132} = 32% above ambient ultraviolet-B, UV-B_{162} = 62% above ambient. - = not determined.

UV-B treatment	N source	Nodule dry matter (g plant^{-1})	Nodule activity (mg N fixed g^{-1}d^{-1})	Root %N	Stem %N	Leaf %N	Pod %N	Total N content (mg plant^{-1})
				Lupinus luteus				
$Visible_{cont.}$	Symbiotic N	0.41	6.28	1.07	2.06	4.59a	2.93	288.3
	NO_3-N	0.32	7.34	0.96	2.14	4.78a	3.02	255.8
$UV\text{-}A_{cont.}$	Symbiotic N	0.55	6.01	1.26	1.83	**4.10b**	3.34	370.5
	NO_3-N	0.31	7.70	1.18	2.11	**4.95a**	3.21	257.8
$UV\text{-}B_{22}$	Symbiotic N	0.47	6.21	1.07	2.06	4.45a	2.97	329.1
	NO_3-N	0.32	7.28	1.22	2.20	4.73a	2.89	266.8
Wald χ^2 statistic								
UV-B (d.f.=2)		0.23	0.16	1.56	4.18	0.95	0.89	0.42
N (d.f.=1)		**14.27*****	**11.77*****	0.03	**6.53***	**29.20*****	0.03	**12.05****
UV-BxN (d.f.=2)		4.49	0.49	0.89	1.82	**11.05****	1.22	4.42
				Vicia atropurpurea				
$Visible_{cont.}$	Symbiotic N	0.18	19.95	**1.97b**	2.42	4.21	-	464.0a
	NO_3-N	0.22	30.57	**2.76a**	2.45	4.32	-	389.2a
$UV\text{-}A_{cont.}$	Symbiotic N	0.22	16.71	2.70a	2.37	3.97	-	419.3a
	NO_3-N	0.13	17.60	2.24a	2.99	4.75	-	275.9a
$UV\text{-}B_{22}$	Symbiotic N	0.27	15.16	2.39a	2.40	4.12	-	574.0a
	NO_3-N	0.19	17.64	2.19a	2.62	4.45	-	240.8a
Wald χ^2 statistic								
UV-B (d.f.=2)		0.22	0.10	0.37	0.44	0.24	-	0.07
N (d.f.=1)		0.46	0.02	0.28	**5.73***	**6.51***	-	2.50
UV-BxN (d.f.=2)		1.33	1.96	**8.69***	4.81	5.44	-	**5.86***

Appendix 6. Effects of below-ambient UV-B radiation on concentration of flavonoids, anthocyanins and non-structural carbohydates in roots or leaves of purely symbiotic and NO_3-fed nodulated plants of *Lupinus luteus* and *Vicia atropurpurea*. Significantly different means within N source treatment at $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{132} = 32% above ambient ultraviolet-B, UV-B_{162} = 62% above ambient.

UV-B Treatment	N source	Root flavanoids (Abs g^{-1})	Root anthocyanins (Abs g^{-1} DM)	Root soluble sugars (mg g^{-1} DM)	Root starch (mg g^{-1} DM)	leaf flavanoids (Abs g^{-1} DM)	Leaf anthocyanins (Abs g^{-1} DM)
				Lupinus luteus			
$Visible_{cont.}$	Symbiotic N	82.36	0.70a	4.06	90.69	215.1	0.58
	NO_3-N	75.85	0.71a	1.39	73.94	204.4	0.82
$UV\text{-}A_{cont.}$	Symbiotic N	92.99	0.85a	3.99	78.20	232.6	0.90
	NO_3-N	75.72	0.62a	1.73	65.30	204.1	0.74
$UV\text{-}B_{22}$	Symbiotic N	88.61	**0.83a**	5.36	85.76	237.8	0.63
	NO_3-N	65.53	**0.58b**	0.86	70.16	212.2	0.76
Wald χ^2 statistic							
UV-B (d.f.=2)		1.65	0.48	0.58	3.44	2.73	1.16
N (d.f.=1)		**13.89*****	**8.57****	**18.10*****	**7.13****	**4.50***	0.27
UV-BxN (d.f.=2)		3.90	**9.29***	0.36	1.11	0.61	2.12
				Vicia atropurpurea			
$Visible_{cont.}$	Symbiotic N	61.72	1.04	7.93a	66.11	109.7	1.40
	NO_3-N	68.44	0.96	17.14a	74.90	102.1	0.44
$UV\text{-}A_{cont.}$	Symbiotic N	57.47	0.96	**16.37a**	74.66	122.1	1.23
	NO_3-N	48.14	0.81	**6.25b**	70.06	88.4	0.44
$UV\text{-}B_{22}$	Symbiotic N	68.92	1.00	11.05a	79.76	105.1	1.16
	NO_3-N	45.23	0.67	5.92a	64.76	104.2	1.00
Wald χ^2 statistic							
UV-B (d.f.=2)		2.38	0.13	3.04	0.37	0.20	0.74
N (d.f.=1)		1.08	1.85	0.85	0.02	1.01	**9.79****
UV-BxN (d.f.=2)		2.05	0.72	**6.26***	1.72	2.07	1.45

Appendix 7. Effects of elevated UV-B radiation on organ or total dry matter accumulation of purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within N source treatment at $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{137} = 37% above ambient ultraviolet-B, UV-B_{173} = 73% above ambient. DM = dry matter.

N source/ UV-B treatment		Plant growth (g plant^{-1}) Leaf DM	Stem DM	Root DM	Nodule DM	Total DM
				Virgilia oroboides		
Main effects:						
	UV-B_{100}	9.85	8.13	10.50	1.88	30.36
	UV-B_{132}	9.11	7.57	9.83	1.80	28.32
	UV-B_{162}	8.63	6.65	9.00	1.73	26.01
	Symbiotic N	**7.78b**	**5.45b**	**8.00b**	**1.62b**	**22.84b**
	NO_3-N	**10.62a**	**9.45a**	**11.55a**	**1.99a**	**33.61a**
Interactions:						
Symbiotic N	UV-B_{100}	1.61	4.67	8.77	1.67	24.22
	UV-B_{132}	1.62	4.11	7.59	1.26	20.45
	UV-B_{162}	2.02	4.82	8.24	1.45	23.83
NO_3-N	UV-B_{100}	0.54	7.84	13.23	1.61	35.70
	UV-B_{137}	1.30	7.08	13.93	1.41	34.00
	UV-B_{173}	1.12	7.42	12.64	1.72	33.90
Wald χ^2 statistic						
UV-B (d.f.=2)		0.83	2.11	0.75	0.78	1.06
N (d.f.=1)		**13.57*** **	**27.03*** **	**19.62*** **	**9.09****	**22.64*** **
UV-BxN (d.f.=2)		1.46	2.45	1.11	0.50	1.69
				Cyclopia genistoides		
Main effects:						
	UV-B_{100}	**10.28a**	**7.09a**	11.08	1.28	29.73
	UV-B_{132}	**8.33ab**	**5.11ab**	9.06	1.32	23.81
	UV-B_{162}	**6.81b**	**4.32b**	9.14	1.16	21.43
	Symbiotic N	8.14	5.15	**8.54b**	**1.38a**	23.20
	NO_3-N	8.86	5.90	**10.95a**	**1.14b**	26.85
Interactions:						
Symbiotic N	UV-B_{100}	9.26	6.24	9.40	1.21	26.11
	UV-B_{137}	8.81	5.49	9.27	1.58	25.14
	UV-B_{173}	6.63	3.85	7.15	1.43	19.07
NO_3-N	UV-B_{100}	11.31	7.93	12.76	1.35	33.35
	UV-B_{137}	8.56	5.19	9.39	1.16	24.29
	UV-B_{173}	7.87	4.96	11.28	0.99	25.10
Wald χ^2 statistic:						
UV-B (d.f.=2)		**5.93***	8.10*	1.25	0.50	4.84
N (d.f.=1)		0.87	2.09	**12.43****	**5.19***	3.42
UV-BxN (d.f.=2)		3.08	**7.69***	**6.83****	**6.84***	**8.16***
				Podalyria calyptrata		
Main effects:						
	UV-B_{100}	10.96	6.16	10.84	1.62	29.58
	UV-B_{132}	9.54	5.59	10.76	1.34	27.22
	UV-B_{162}	10.70	6.06	10.30	1.54	28.59
	Symbiotic N	**8.57b**	**4.47b**	**8.09b**	1.45	**22.58b**
	NO_3-N	**12.23a**	**7.41a**	**13.17a**	1.54	**34.35a**
Interactions:						
Symbiotic N	UV-B_{100}	9.11	4.67	8.77	1.67	24.22
	UV-B_{137}	7.49	4.11	7.59	1.26	20.45
	UV-B_{173}	9.31	4.82	8.24	1.45	23.83
NO_3-N	UV-B_{100}	13.03	7.84	13.23	1.61	35.70
	UV-B_{137}	11.58	7.08	13.93	1.41	34.00
	UV-B_{173}	12.12	7.42	12.64	1.72	33.90
Wald χ^2 statistic:						
UV-B (d.f.=2)		1.09	0.69	0.33	1.54	0.64
N (d.f.=1)		**13.48*** **	**18.45*** **	**38.91*** **	0.97	**21.06*** **
UV-BxN (d.f.=2)		0.17	0.11	0.86	0.54	0.28

Appendix 8. Effects of elevated UV-B radiation on symbiotic parameters of purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{132} = 37% above ambient ultraviolet-B, UV-B_{173} = 73% above ambient.

N source/UV-B treatment		Nodule %N	Root %N	Stem %N	Leaf %N	Total N content (mg plant^{-1})
				Virgilia oroboides		
Main effects:						
	UV-B_{100}	4.72	1.84	1.15	**2.55a**	635.08
	UV-B_{137}	4.76	1.81	1.14	**2.67a**	598.27
	UV-B_{173}	4.40	1.75	1.07	**2.25b**	509.60
	Symbiotic N	4.76	1.76	1.10	2.39	**476.52b**
	NO_3-N	4.49	1.83	1.14	2.59	**685.44a**
Interactions:						
Symbiotic N	UV-B_{100}	4.66	1.80	1.14	2.39	484.10
	UV-B_{137}	5.15	1.80	1.08	2.70	542.03
	UV-B_{173}	4.48	1.69	1.07	2.073	403.45
NO_3-N	UV-B_{100}	4.79	1.88	1.15	2.72	786.06
	UV-B_{137}	4.38	1.82	1.20	2.63	654.51
	UV-B_{173}	4.32	1.80	1.06	2.43	615.75
Wald χ^2 statistic:						
UV-B (d.f.=2)		2.01	0.79	1.39	**6.54***	2.34
N (d.f.=1)		1.16	0.71	0.54	2.69	**14.74*****
UV-BxN (d.f.=2)		2.92	0.13	0.75	2.59	1.60
				Cyclopia genistoides		
Main effects:						
	UV-B_{100}	4.14	1.83	1.43	2.69	632.70
	UV-B_{137}	3.73	1.73	1.36	2.60	498.33
	UV-B_{173}	3.54	1.78	1.28	2.58	429.68
	Symbiotic N	**3.65b**	1.90	**1.43a**	**2.88a**	522.08
	NO_3-N	**3.96a**	1.67	**1.29b**	**2.38b**	522.24
Interactions:						
Symbiotic N	UV-B_{100}	3.79	1.82	1.51	2.90	580.21
	UV-B_{137}	3.84	2.01	1.37	2.89	577.20
	UV-B_{173}	3.31	1.94	1.41	2.83	427.24
NO_3-N	UV-B_{100}	4.50	1.85	1.36	2.47	685.18
	UV-B_{137}	3.70	1.48	1.38	2.40	459.06
	UV-B_{173}	3.89	1.71	1.14	2.38	472.19
Wald χ^2 statistic:						
UV-B (d.f.=2)		5.18	0.49	1.65	0.78	4.85
N (d.f.=1)		**3.99***	**2.51**	**4.13***	**16.61*****	0.00
UV-BxN (d.f.=2)		**8.57***	2.34	2.33	0.61	**8.68***
				Podalyria calyptrata		
Mian effects:						
	UV-B_{100}	4.53	1.77	1.15	1.83	539.42
	UV-B_{137}	4.44	1.64	1.13	2.09	506.92
	UV-B_{173}	4.70	1.73	1.17	1.98	542.65
	Symbiotic N	4.64	1.74	1.15	2.03	**446.38b**
	NO_3-N	4.47	1.69	1.15	1.92	**612.94a**
Interactions:						
Symbiotic N	UV-B_{100}	4.41	1.78	1.17	2.02	478.17
	UV-B_{137}	4.62	1.73	1.16	2.06	401.25
	UV-B_{173}	4.20	1.69	1.15	2.05	476.79
NO_3-N	UV-B_{100}	4.62	1.73	1.15	1.75	617.73
	UV-B_{137}	4.20	1.56	1.10	2.12	612.59
	UV-B_{173}	4.60	1.66	1.14	1.93	607.10
Wald χ^2 tatistic:						
UV-B (d.f.=2)		0.98	0.91	0.55	1.73	0.51
N (d.f.=1)		0.56	0.13	0.00	1.23	**11.15*****
UV-BxN (d.f.=2)		2.21	1.14	0.68	1.50	0.24

Appendix 9. Effects of elevated UV-B radiation on concentration of flavonoids, anthocyanins and non-structural carbohydrates in roots or leaves of purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. $UV\text{-}B_{100}$ = ambient ultraviolet-B radiation, $UV\text{-}B_{137}$ = 32% above ambient ultraviolet-B, $UV\text{-}B_{173}$ = 73% above ambient. - = not determined, DM = dry matter.

N source/UV-B treatment		Root flavanoids (Abs g^{-1} DM)	Root anthocyanins (Abs g^{-1} DM)	Root soluble sugars (mg g^{-1} DM)	Root starch (mg g^{-1} DM)	leaf flavanoids (Abs g^{-1} DM)	Leaf anthocyanins (Abs g^{-1} DM)
				Virgilia oroboides			
Main effects:							
	$UV\text{-}B_{100}$	57.93	0.97	20.99	102.13	94.60	0.79
	$UV\text{-}B_{137}$	58.32	0.98	25.44	105.20	96.67	0.78
	$UV\text{-}B_{173}$	63.80	0.88	25.16	97.25	95.85	0.86
	Symbiotic N	58.52	0.93	**21.63b**	97.26	**91.24b**	0.85
	NO_3-N	61.51	0.95	**26.09a**	105.80	**100.18a**	0.76
Interactions:							
Symbiotic N	$UV\text{-}B_{100}$	55.90	0.92	19.63	96.71	82.25	0.89
	$UV\text{-}B_{137}$	57.17	0.98	23.47	98.61	99.41	0.73
	$UV\text{-}B_{173}$	62.48	0.89	21.80	96.41	92.05	0.94
NO_3-N	$UV\text{-}B_{100}$	59.95	1.01	22.35	107.55	-	0.70
	$UV\text{-}B_{137}$	59.46	0.98	27.40	111.74	93.93	0.82
	$UV\text{-}B_{173}$	65.13	0.86	28.52	98.10	99.65	0.77
Wald χ^2 statistic:							
UV-B (d.f.=2)		0.26	4.50	2.21	1.77	0.08	0.43
N (d.f.=1)		0.73	0.14	**4.61***	3.69	**7.01***	0.05
UV-BxN (d.f.=2)		0.05	1.26	0.63	1.59	**10.12****	1.89
				Cyclopia genistoides			
Main effect:							
	$UV\text{-}B_{100}$	152.31	1.38	31.61	88.38	455.26	0.39
	$UV\text{-}B_{137}$	163.26	1.53	34.04	89.92	464.12	0.39
	$UV\text{-}B_{173}$	154.56	1.33	27.33	83.27	498.52	0.32
	Symbiotic N	160.65	**1.53a**	31.01	88.51	480.64	**0.42a**
	NO_3-N	153.03	**1.30b**	31.13	86.09	462.38	**0.31b**
Interactions:							
Symbiotic N	$UV\text{-}B_{100}$	158.47	1.52	30.76	87.38	453.43	0.40
	$UV\text{-}B_{137}$	178.80	1.66	35.81	92.41	490.76	0.44
	$UV\text{-}B_{173}$	135.58	1.40	26.90	87.80	494.23	0.40
NO_3-N	$UV\text{-}B_{100}$	146.16	1.23	32.47	89.38	457.10	0.37
	$UV\text{-}B_{137}$	152.64	1.42	29.83	87.34	437.89	0.27
	$UV\text{-}B_{173}$	173.09	1.28	29.64	80.97	496.86	0.22
Wald χ^2 statistic:							
UV-B (d.f.=2)		0.17	2.01	0.51	5.69	1.71	4.51
N (d.f.=1)		0.80	**8.31***	0.28	1.70	1.63	**6.65***
UV-BxN (d.f.=2)		**11.42****	0.90	0.83	3.60	4.50	**6.64***
				Podalyria calyptrata			
Main effects:							
	$UV\text{-}B_{100}$	82.09	0.63	17.95	118.14	179.05	0.00
	$UV\text{-}B_{137}$	73.22	0.57	15.34	122.60	179.49	0.00
	$UV\text{-}B_{173}$	81.06	0.68	19.50	119.63	182.64	0.00
	Symbiotic N	82.53	0.65	17.22	125.34	**189.00a**	0.00
	NO_3-N	75.05	0.60	17.97	114.90	**171.75b**	0.00
Interactions:							
Symbiotic N	$UV\text{-}B_{100}$	85.48	0.63	19.87	124.32	190.31	0.00
	$UV\text{-}B_{137}$	78.74	0.57	12.45	120.23	177.16	0.00
	$UV\text{-}B_{173}$	74.50	0.74	18.89	131.00	199.70	0.00
NO_3-N	$UV\text{-}B_{100}$	78.74	0.63	15.57	111.49	169.20	0.00
	$UV\text{-}B_{137}$	71.94	0.56	18.23	124.97	181.82	0.00
	$UV\text{-}B_{173}$	74.46	0.59	20.89	107.38	161.54	0.00
Wald χ^2 statistic:							
UV-B (d.f.=2)		0.77	2.48	3.48	0.08	0.08	-
N (d.f.=1)		2.88	1.33	0.00	2.28	**3.89***	-
UV-BxN (d.f.=2)		0.52	0.95	**6.28***	5.43	3.75	-

Appendix 10. Effects of below-ambient UV-B radiation on organ or total dry matter accumulation of purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within N source treatment at $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ in bold type and separated by different letters. UV-B_{22} = 22% of ambient ultraviolet-B radiation, UV-$A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control. DM = dry matter.

N source/ UV-B treatment		Plant growth (g plant^{-1}) Leaf DM	Stem DM	Root DM	Nodule DM	Total DM
				Virgilia oroboides		
Main effects:						
	$PAR_{cont.}$	5.37	5.41	6.67	0.97	18.42
	UV-$A_{cont.}$	5.26	5.29	7.85	0.88	19.28
	UV-B_{22}	4.44	4.55	6.25	0.96	16.21
	Symbiotic N	4.45	**4.08b**	**6.10b**	0.90	**15.53b**
	NO_3-N	5.60	**9.45a**	**7.75a**	0.97	**20.41a**
Interactions:						
Symbiotic N	$PAR_{cont.}$	3.96	3.35	5.42	0.88	13.71
	UV-$A_{cont.}$	4.86	4.37	6.91	0.78	16.92
	UV-B_{22}	4.53	4.43	5.97	1.03	15.96
NO_3-N	$PAR_{cont.}$	6.79	7.37	7.92	1.05	23.12
	UV-$A_{cont.}$	5.65	6.22	8.79	0.99	21.65
	UV-B_{22}	4.35	4.68	6.54	0.89	16.46
Wald χ^2 statistic:						
UV-B (d.f.=2)		1.95	1.22	2.72	0.04	1.90
N (d.f.=1)		3.45	**8.44****	**7.12****	0.56	**9.05****
UV-BxN (d.f.=2)		4.40	3.92	1.01	2.70	4.59
				Cyclopia genistoides		
Main effects:						
	$PAR_{cont.}$	4.48	2.57	4.95	0.46	12.62
	UV-$A_{cont.}$	5.01	3.62	4.99	0.50	14.12
	UV-B_{22}	3.96	3.24	4.71	0.47	12.37
	Symbiotic N	4.47	3.34	4.75	**0.56a**	13.12
	NO_3-N	4.50	3.01	5.00	**0.39b**	13.01
Interactions:						
Symbiotic N	$PAR_{cont.}$	3.38	2.60	3.92	0.57	10.47
	UV-$A_{cont.}$	5.93	4.17	5.56	0.58	16.24
	UV-B_{22}	3.89	3.30	4.50	0.55	12.23
NO_3-N	$PAR_{cont.}$	5.66	2.75	5.87	0.38	14.66
	UV-$A_{cont.}$	4.09	3.08	4.42	0.42	12.00
	UV-B_{22}	4.03	3.17	4.92	0.39	12.52
Wald χ^2 statistic:						
UV-B (d.f.=2)		1.15	2.66	0.14	0.40	0.78
N (d.f.=1)		0.31	0.02	0.31	**8.90****	0.03
UV-BxN (d.f.=2)		**11.02***	3.38	**5.92***	0.09	**6.45***
				Podalyria calyptrata		
Main effects:						
	$PAR_{cont.}$	4.87	2.28	5.71	0.67	13.53
	UV-$A_{cont.}$	6.21	3.39	7.18	0.70	17.47
	UV-B_{22}	4.44	2.23	5.71	0.56	12.94
	Symbiotic N	4.98	2.66	**5.62b**	**0.74a**	14.00
	NO_3-N	5.37	2.61	**6.78a**	**0.545**	15.30
Interactions:						
Symbiotic N	$PAR_{cont.}$	3.56	1.73	4.48	0.66	10.42
	UV-$A_{cont.}$	7.21	4.07	7.12	0.95	19.35
	UV-B_{22}	5.17	2.76	5.88	0.66	14.47
NO_3-N	$PAR_{cont.}$	6.57	2.88	7.22	0.66	17.33
	UV-$A_{cont.}$	6.12	3.29	8.43	0.55	18.39
	UV-B_{22}	4.63	2.27	5.89	0.54	13.33
Wald χ^2 statistic						
UV-B (d.f.=2)		1.69	1.38	2.40	0.52	1.95
N (d.f.=1)		0.46	0.06	**3.69***	**6.07***	0.89
UV-BxN (d.f.=2)		**6.81***	5.11	4.12	**6.77***	**7.15***

Appendix 11. Effects of below-ambient UV-B radiation on symbiotic parameters of purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{22} = 22% of ambient ultraviolet-B radiation, UV-$A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control.

N source/UV-B treament		Nodule activity (mg N fixed $g^{-1}d^{-1}$)	Nodule %N	Root %N	Stem %N	Leaf %N	Total N content (mg $plant^{-1}$)
				Virgilia oroboides			
Main effects:							
	$PAR_{cont.}$	1.91	5.03	1.60	1.25	3.24	407.46
	UV-$A_{cont.}$	2.20	5.71	1.75	1.26	3.58	442.50
	UV-B_{22}	1.56	5.17	1.60	1.28	3.10	344.43
	Symbiotic N	**1.678**	5.24	1.61	1.30	3.19	**339.68b**
	NO_3-N	**2.10a**	5.36	1.69	1.14	3.42	**456.68a**
Interactions:							
Symbiotic N	$PAR_{cont.}$	2.04	4.85	1.45	1.26	3.07	285.99
	UV-$A_{cont.}$	3.17	5.64	1.69	1.31	3.39	383.38
	UV-B_{22}	2.15	5.25	1.68	1.34	3.12	349.38
NO_3-N	$PAR_{cont.}$	3.54	5.22	1.76	1.236	3.42	528.93
	UV-$A_{cont.}$	3.26	5.79	1.80	1.215	3.77	501.62
	UV-B_{22}	2.41	5.09	1.52	1.221	3.08	339.49
Wald χ^2 statistic:							
UV-B (d.f.=2)		3.73	3.50	2.71	0.12	3.24	2.97
N (d.f.=1)		**6.26***	0.79	2.22	2.29	3.23	**9.20****
UV-BxN (d.f.=2)		3.96	3.17	**11.73****	0.81	1.43	**8.43***
				Cyclopia genistoides			
Main effects:							
	$PAR_{cont.}$	2.56	**4.27b**	1.48	**0.97b**	**2.23b**	229.11
	UV-$A_{cont.}$	2.72	**4.86a**	1.85	**1.12a**	**2.81a**	295.97
	UV-B_{22}	2.41	**4.28b**	1.91	**1.06ab**	**2.63a**	254.51
	Symbiotic N	**2.01b**	**4.25b**	**1.76b**	1.09	**2.84a**	271.29
	NO_3-N	**3.10a**	**4.70a**	**1.92a**	1.02	**2.33b**	252.94
Interactions:							
Symbiotic N	$PAR_{cont.}$	1.80	4.10	1.61	1.07	2.75	201.39
	UV-$A_{cont.}$	3.15	4.63	1.79	1.15	2.95	341.83
	UV-B_{22}	2.41	4.14	1.87	1.07	2.96	263.46
NO_3-N	$PAR_{cont.}$	4.43	4.37	1.88	0.90	1.91	257.82
	UV-$A_{cont.}$	3.59	5.09	1.90	1.08	2.68	250.10
	UV-B_{22}	3.56	4.42	1.94	1.06	2.29	245.55
Wald χ^2 statistic:							
UV-B (d.f.=2)		2.10	**8.87***	1.54	**6.16***	**11.54****	1.98
N (d.f.=1)		**16.46*****	**5.31***	**6.01***	3.33	**14.77*****	0.05
UV-BxN (d.f.=2)		**10.76****	0.88	3.82	1.35	1.92	**6.60***
				Podalyria calyptrata			
Main effects:							
	$PAR_{cont.}$	**1.78b**	4.40	2.19	**1.19b**	1.92	275.56
	UV-$A_{cont.}$	**2.41a**	4.78	2.29	**1.35a**	2.14	387.59
	UV-B_{22}	**1.98b**	4.47	2.00	**1.19b**	2.07	259.88
	Symbiotic N	**1.65b**	4.58	2.15	1.25	2.03	297.71
	NO_3-N	**2.46a**	4.52	2.16	1.23	2.05	317.64
Ineractions:							
Symbiotic N	$PAR_{cont.}$	1.93	4.31	2.22	1.18	1.94	215.44
	UV-$A_{cont.}$	2.52	4.88	2.28	1.38	2.08	426.89
	UV-B_{22}	2.38	4.94	1.97	1.25	2.02	300.80
NO_3-N	$PAR_{cont.}$	3.05	4.76	2.16	1.25	1.97	354.31
	UV-$A_{cont.}$	4.14	4.86	2.40	1.30	2.21	416.78
	UV-B_{22}	3.06	4.46	2.01	1.12	2.10	260.82
Wald χ^2 statistic:							
UV-B (d.f.=2)		**7.16***	1.25	1.18	**6.02***	1.01	2.06
N (d.f.=1)		**19.86*****	0.18	0.02	0.07	0.04	0.54
UV-BxN (d.f.=2)		3.19	1.07	1.67	2.14	0.52	5.40

Appendix 12. Effects of below-ambient UV-B radiation on concentration of flavonoids, anthocyanins and non-structural carbohydates in roots or leaves of purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{22} = 22% of ambient ultraviolet-B radiation, UV-$A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control. - = not determined, DM = dry matter.

N source/UV-B treatment		Root flavanoids (Abs g^{-1})	Root anthocyanins (Abs g^{-1} DM)	Root soluble sugars (mg g^{-1} DM)	Root starch (mg g^{-1} DM)	leaf flavanoids (Abs g^{-1} DM)	Leaf anthocyanins (Abs g^{-1} DM)
				Virgilia oroboides			
Main effects:							
	$PAR_{cont.}$	61.31	0.98	13.80	99.75	112.09	0.85
	UV-$A_{cont.}$	52.93	0.89	13.49	100.41	104.84	0.85
	UV-B_{22}	50.59	0.87	15.04	99.82	101.38	0.72
	Symbiotic N	**52.32b**	0.94	**12.28b**	96.68	106.34	0.81
	NO_3-N	**57.56a**	0.89	**15.94a**	103.31	105.85	0.80
Interactions:							
Symbiotic N	$PAR_{cont.}$	63.82	0.98	12.70	97.31	114.93	0.83
	UV-$A_{cont.}$	45.94	0.90	12.25	91.43	104.96	0.93
	UV-B_{22}	47.20	0.94	11.87	101.40	99.132	0.68
NO_3-N	$PAR_{cont.}$	58.80	0.98	14.90	102.19	109.25	0.86
	UV-$A_{cont.}$	59.91	0.89	14.71	109.40	104.73	0.77
	UV-B_{22}	53.98	0.80	18.20	98.35	103.62	0.75
Wald χ^2 statistic:							
UV-B (d.f.=2)		4.44	3.74	0.14	0.06	5.43	0.83
N (d.f.=1)		**7.69****	1.20	**7.54****	2.43	0.02	0.05
UV-BxN (d.f.=2)		3.65	1.79	1.28	4.83	0.87	2.90
				Cyclopia genistoides			
Main effects:							
	$PAR_{cont.}$	212.86	35.55	30.88	123.52	475.87	0.52
	UV-$A_{cont.}$	189.24	33.35	26.43	121.66	503.08	0.31
	UV-B_{22}	206.44	36.13	22.71	116.82	467.53	0.42
	Symbiotic N	**220.00a**	**37.01a**	27.55	122.12	487.62	0.42
	NO_3-N	**185.14b**	**33.07b**	25.66	119.16	477.68	0.41
Interactions:							
Symbiotic N	$PAR_{cont.}$	260.72	35.79	26.86	92.412	485.85	0.45
	UV-$A_{cont.}$	196.08	34.86	33.39	96.214	490.33	0.36
	UV-B_{22}	210.70	39.24	19.02	95.557	474.83	0.39
NO_3-N	$PAR_{cont.}$	171.22	32.72	31.87	94.036	467.23	0.61
	UV-$A_{cont.}$	182.40	31.85	19.48	94.244	515.83	0.26
	UV-B_{22}	202.18	33.02	26.40	92.670	474.83	0.45
Wald χ^2 statistic:							
UV-B (d.f.=2)		2.43	2.07	1.83	0.39	2.17	1.81
N (d.f.=1)		**7.84***	**4.33***	0.02	2.48	0.18	0.18
UV-BxN (d.f.=2)		**6.93***	0.43	4.04	4.33	2.31	3.46
				Podalyria calyptrata			
Main effects:							
	$PAR_{cont.}$	53.00	0.49	13.59	86.20	**121.83b**	0.00
	UV-$A_{cont.}$	53.37	0.50	13.16	73.47	**115.03b**	0.00
	UV-B_{22}	54.24	0.56	13.98	74.53	**142.47a**	0.00
	Symbiotic N	58.48	**0.57a**	**15.79a**	82.30	127.94	0.00
	NO_3-N	48.77	**0.47b**	**11.36b**	73.83	125.37	0.00
Interactions:							
Symbiotic N	$PAR_{cont.}$	58.56	0.54	14.73	89.64	121.17	0.00
	UV-$A_{cont.}$	61.29	0.52	15.84	79.12	115.74	0.00
	UV-B_{22}	59.07	0.68	15.88	86.16	138.63	0.00
NO_3-N	$PAR_{cont.}$	48.24	0.46	11.44	81.52	117.78	0.00
	UV-$A_{cont.}$	46.68	0.51	10.75	71.04	112.87	0.00
	UV-B_{22}	53.82	0.44	11.79	66.93	139.65	0.00
Wald χ^2 statistic:							
UV-B (d.f.=2)		0.08	1.46	0.19	2.43	**31.60*****	-
N (d.f.=1)		2.16	**5.37***	**8.84***	3.65	1.10	-
UV-BxN (d.f.=2)		1.25	1.27	0.34	0.04	1.10	-

Appendix 13. Effects of elevated UV-B radiation on organ or total dry matter accumulation of purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within N source treatment at $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{137} = 37% above ambient ultraviolet-B, UV-B_{173} = 73% above ambient. DM = dry matter.

UV-B treatment	N source	Plant growth (g plant^{-1})				
		Leaf DM	Stem DM	Root DM	Nodule DM	Total DM
				Virgilia oroboides		
UV-B_{100}	Symbiotic N	1.61	4.67	8.77	1.67	24.22
	NO_3	0.54	7.84	13.23	1.61	35.70
UV-B_{137}	Symbiotic N	1.62	4.11	7.59	1.26	20.45
	NO_3	1.30	7.08	13.93	1.41	34.00
UV-B_{173}	Symbiotic N	2.02	4.82	8.24	1.45	23.83
	NO_3	1.12	7.42	12.64	1.72	33.90
Wald χ^2 statistic						
UV-B (d.f.=2)		0.83	2.11	0.75	0.78	1.06
N (d.f.=1)		**13.57*** **	**27.03*** **	**19.62*** **	**9.09****	**22.64*** **
UV-BxN (d.f.=2)		1.46	2.45	1.11	0.50	1.69
				Cyclopia genistoides		
UV-B_{100}	Symbiotic N	9.26	6.24a	9.40a	1.21a	26.11a
	NO_3	11.31	7.93a	12.76a	1.35a	33.35a
UV-B_{137}	Symbiotic N	8.81	5.49a	9.27a	1.58a	25.14a
	NO_3	8.56	5.19a	9.39a	1.16a	24.29a
UV-B_{173}	Symbiotic N	6.63	3.85a	7.15a	1.43a	**19.07b**
	NO_3	7.87	4.96a	11.28a	0.99a	**25.10a**
Wald χ^2 statistic:						
UV-B (d.f.=2)		**5.93***	8.10*	1.25	0.50	4.84
N (d.f.=1)		0.87	2.09	**12.43****	**5.19***	3.42
UV-BxN (d.f.=2)		3.08	**7.69***	**6.83****	**6.84***	**8.16***
				Podalyria calyptrata		
UV-B_{100}	Symbiotic N	9.11	4.67	8.77	1.67	24.22
	NO_3	13.03	7.84	13.23	1.61	35.70
UV-B_{137}	Symbiotic N	7.49	4.11	7.59	1.26	20.45
	NO_3	11.58	7.08	13.93	1.41	34.00
UV-B_{173}	Symbiotic N	9.31	4.82	8.24	1.45	23.83
	NO_3	12.12	7.42	12.64	1.72	33.90
Wald χ^2 statistic:						
UV-B (d.f.=2)		1.09	0.69	0.33	1.54	0.64
N (d.f.=1)		**13.48*** **	**18.45*** **	**38.91*** **	0.97	**21.06*** **
UV-BxN (d.f.=2)		0.17	0.11	0.86	0.54	0.28

Appendix 14. Effects of elevated UV-B radiation on symbiotic parameters of purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{132} = 37% above ambient ultraviolet-B, UV-B_{173} = 73% above ambient.

UV-B treatment	N source	Nodule %N	Root %N	Stem %N	Leaf %N	Total N content (mg plant^{-1})
			Virgilia oroboides			
UV-B_{100}	Symbiotic N	4.66	1.80	1.14	2.39	484.10
	NO_3	4.79	1.88	1.15	2.72	786.06
UV-B_{137}	Symbiotic N	5.15	1.80	1.08	2.70	542.03
	NO_3	4.38	1.82	1.20	2.63	654.51
UV-B_{173}	Symbiotic N	4.48	1.69	1.07	2.07	403.45
	NO_3	4.32	1.80	1.06	2.43	615.75
Wald χ^2 statistic:						
UV-B (d.f.=2)		2.01	0.79	1.39	**6.54***	2.34
N (d.f.=1)		1.16	0.71	0.54	2.69	**14.74*****
UV-BxN (d.f.=2)		2.92	0.13	0.75	2.59	1.60
			Cyclopia genistoides			
UV-B_{100}	Symbiotic N	**3.79b**	1.82	1.51	2.90	580.21a
	NO_3	**4.50a**	1.85	1.36	2.47	685.18a
UV-B_{137}	Symbiotic N	3.84a	2.01	1.37	2.89	577.20a
	NO_3	3.70a	1.48	1.38	2.40	459.06a
UV-B_{173}	Symbiotic N	3.31a	1.94	1.41	2.83	427.24a
	NO_3	3.89a	1.71	1.14	2.38	472.19a
Wald χ^2 statistic:						
UV-B (d.f.=2)		5.18	0.49	1.65	0.78	4.85
N (d.f.=1)		**3.99***	**2.51**	**4.13***	**16.61*****	0.00
UV-BxN (d.f.=2)		**8.57***	2.34	2.33	0.61	**8.68***
			Podalyria calyptrate			
UV-B_{100}	Symbiotic N	**4.41**	1.78	1.17	2.02	478.17
	NO_3	4.62	1.73	1.15	1.75	617.73
UV-B_{137}	Symbiotic N	4.62	1.73	1.16	2.06	401.25
	NO_3	4.20	1.56	1.10	2.12	612.59
UV-B_{173}	Symbiotic N	4.20	1.69	1.15	2.05	476.79
	NO_3	4.60	1.66	1.14	1.93	607.10
Wald χ^2 tatistic:						
UV-B (d.f.=2)		0.98	0.91	0.55	1.73	0.51
N (d.f.=1)		0.56	0.13	0.00	1.23	**11.15*****
UV-BxN (d.f.=2)		2.21	1.14	0.68	1.50	0.24

Appendix 15. Effects of elevated UV-B radiation on concentration of flavonoids, anthocyanins and non-structural carbohydrates in roots or leaves of purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{100} = ambient ultraviolet-B radiation, UV-B_{137} = 32% above ambient ultraviolet-B, UV-B_{173} = 73% above ambient. DM = dry matter.

UV-B treatment	N source	Root flavanoids (Abs g^{-1} DM)	Root anthocyanins (Abs g^{-1} DM)	Root soluble sugars (mg g^{-1} DM)	Root starch (mg g^{-1} DM)	leaf flavanoids (Abs g^{-1} DM)	Leaf anthocyanins (Abs g^{-1} DM)
				Virgilia oroboides			
:							
UV-B_{100}	Symbiotic N	55.90	0.92	19.63	96.71	82.25a	0.89
	NO_3	59.95	1.01	22.35	107.55	106.27a	0.70
UV-B_{137}	Symbiotic N	57.17	0.98	23.47	98.67	99.41a	0.73
	NO_3	59.46	0.98	27.40	111.74	93.93a	0.82
UV-B_{173}	Symbiotic N	62.48	0.89	21.80	96.41	92.05a	0.94
	NO_3	65.13	0.86	28.52	98.10	99.65a	0.77
Wald χ^2 statistic:							
UV-B (d.f.=2)		0.26	4.50	2.21	1.77	0.08	0.43
N (d.f.=1)		0.73	0.14	**4.61***	3.69	**7.01***	0.05
UV-BxN (d.f.=2)		0.05	1.26	0.63	1.59	**10.12****	1.89
				Cyclopia genistoides			
UV-B_{100}	Symbiotic N	158.47a	1.52	30.76	87.38	453.43	0.40a
	NO_3	146.16a	1.23	32.47	89.38	457.10	0.37a
UV-B_{137}	Symbiotic N	178.80a	1.66	35.81	92.41	490.76	0.44a
	NO_3	152.64a	1.42	29.83	87.34	437.89	0.27a
UV-B_{173}	Symbiotic N	135.58a	1.40	26.90	87.80	494.23	**0.40a**
	NO_3	173.09a	1.28	29.64	80.97	496.86	**0.22b**
Wald χ^2 statistic:							
UV-B (d.f.=2)		0.17	2.01	0.51	5.69	1.71	4.51
N (d.f.=1)		0.80	**8.31***	0.28	1.70	1.63	**6.65***
UV-BxN (d.f.=2)		**11.42****	0.90	0.83	3.60	4.50	**6.64***
:				*Podalyria calyptrate*			
UV-B_{100}	Symbiotic N	85.48	0.63	19.87a	124.32	190.31	0.00
	NO_3	78.74	0.63	15.57a	111.49	169.20	0.00
UV-B_{137}	Symbiotic N	78.74	0.57	12.45a	120.23	177.16	0.00
	NO_3	71.94	0.56	18.23a	124.97	181.82	0.00
UV-B_{173}	Symbiotic N	74.50	0.74	18.89a	131.00	199.70	0.00
	NO_3	74.46	0.59	20.89a	107.38	161.54	0.00
Wald χ^2 statistic:							
UV-B (d.f.=2)		0.77	2.48	3.48	0.08	0.08	-
N (d.f.=1)		2.88	1.33	0.00	2.28	**3.89***	-
UV-BxN (d.f.=2)		0.52	0.95	**6.28***	5.43	3.75	-

Appendix 16. Effects of below-ambient UV-B radiation on organ or total dry matter accumulation of purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{22} = 22% of ambient ultraviolet-B radiation, UV-$A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control. DM = dry matter.

UV-B treatment	N source	Plant growth (g plant^{-1})				
		Leaf DM	Stem DM	Root DM	Nodule DM	Total DM
				Virgilia oroboides		
$PAR_{cont.}$	Symbiotic N	3.96	3.35	5.42	0.88	13.71
	NO_3	6.79	7.37	7.92	1.05	23.12
UV-$A_{cont.}$	Symbiotic N	4.86	4.37	6.91	0.78	16.92
	NO_3	5.65	6.22	8.79	0.99	21.65
UV-B_{22}	Symbiotic N	4.53	4.43	5.97	1.03	15.96
	NO_3	4.35	4.68	6.54	0.89	16.46
Wald χ^2 statistic:						
UV-B (d.f.=2)		1.95	1.22	2.72	0.04	1.90
N (d.f.=1)		3.45	**8.44****	**7.12****	0.56	**9.05****
UV-BxN (d.f.=2)		4.40	3.92	1.01	2.70	4.59
				Cyclopia genistoides		
:						
$PAR_{cont.}$	Symbiotic N	3.38b	2.60	3.92a	0.57	**10.47b**
	NO_3	5.66a	2.75	5.87a	0.38	14.66
UV-$A_{cont.}$	Symbiotic N	5.93a	4.17	5.56a	0.58	**16.24a**
	NO_3	4.09a	3.08	4.42a	0.42	12.00
UV-B_{22}	Symbiotic N	3.89a	3.30	4.50a	0.55	**12.23ab**
	NO_3	4.03a	3.17	4.92a	0.39	12.51
Wald χ^2 statistic:						
UV-B (d.f.=2)		1.15	2.66	0.14	0.40	0.78
N (d.f.=1)		0.31	0.02	0.31	**8.90****	0.03
UV-BxN (d.f.=2)		**11.02***	3.38	**5.92***	0.09	**6.45***
				Podalyria calyptrate		
:						
$PAR_{cont.}$	Symbiotic N	3.56a	1.73	4.48	0.66a	10.42a
	NO_3	6.57a	2.88	7.22	0.66a	17.33a
UV-$A_{cont.}$	Symbiotic N	7.21a	4.07	7.12	**0.95a**	19.35a
	NO_3	6.12a	3.29	8.43	**0.55b**	18.39a
UV-B_{22}	Symbiotic N	5.17a	2.76	5.88	0.66a	14.47a
	NO_3	4.63a	2.27	5.89	0.54a	13.33a
Wald χ^2 statistic						
UV-B (d.f.=2)		1.69	1.38	2.40	0.52	1.95
N (d.f.=1)		0.46	0.06	**3.69***	**6.07***	0.89
UV-BxN (d.f.=2)		**6.81***	5.11	4.12	**6.77***	**7.15***

Appendix 17. Effects of below-ambient UV-B radiation on symbiotic parameters of purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within N source treatment at *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 in bold type and separated by different letters. UV-B_{22} = 22% of ambient ultraviolet-B radiation, UV-$A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control.

UV-B treatment	N source	Nodule %N	Root %N	Stem %N	Leaf %N	Total N content (mg plant^{-1})
			Virgilia oroboides			
$PAR_{cont.}$	Symbiotic N	4.85	**1.45b**	1.26	3.07	**285.99b**
	NO_3	5.22	**1.76a**	1.24	3.42	**528.93a**
UV-$A_{cont.}$	Symbiotic N	5.64	1.69a	1.31	3.39	383.38a
	NO_3	5.79	1.80a	1.22	3.77	501.62a
UV-B_{22}	Symbiotic N	5.25	1.68a	1.34	3.12	349.38a
	NO_3	5.09	1.52a	1.22	3.08	339.49a
Wald χ^2 statistic:						
UV-B (d.f.=2)		3.50	2.71	0.12	3.24	2.97
N (d.f.=1)		0.79	2.22	2.29	3.23	**9.20****
UV-BxN (d.f.=2)		3.17	**11.73****	0.81	1.43	**8.43***
			Cyclopia genistoides			
$PAR_{cont.}$	Symbiotic N	4.10	1.61	1.07	2.75	201.39a
	NO_3	4.37	1.88	0.90	1.91	257.82a
UV-$A_{cont.}$	Symbiotic N	4.63	1.79	1.15	2.95	341.83a
	NO_3	5.09	1.90	1.08	2.68	250.10a
UV-B_{22}	Symbiotic N	4.14	1.87	1.07	2.96	263.46a
	NO_3	4.42	1.94	1.06	2.29	245.55a
Wald χ^2 statistic:						
UV-B (d.f.=2)		**8.87***	1.54	**6.16***	**11.54****	1.98
N (d.f.=1)		**5.31***	**6.01***	3.33	**14.77*****	0.05
UV-BxN (d.f.=2)		0.88	3.82	1.35	1.92	**6.60***
			Podalyria calyptrate			
$PAR_{cont.}$	Symbiotic N	4.31	2.22	1.18	1.94	215.44
	NO_3	4.76	2.16	1.25	1.97	354.31
UV-$A_{cont.}$	Symbiotic N	4.88	2.28	1.38	2.08	426.89
	NO_3	4.86	2.40	1.30	2.21	416.78
UV-B_{22}	Symbiotic N	4.95	1.97	1.25	2.02	300.80
	NO_3	4.46	2.01	1.12	2.09	260.82
Wald χ^2 statistic:						
UV-B (d.f.=2)		1.25	1.18	**6.02***	1.01	2.06
N (d.f.=1)		0.18	0.02	0.07	0.04	0.54
UV-BxN (d.f.=2)		1.07	1.67	2.14	0.52	5.40

Appendix 18. Effects of below-ambient UV-B radiation on concentration of flavonoids, anthocyanins and non-structural carbohydates in roots or leaves of purely symbiotic and NO_3-fed nodulated plants of tree and shrub legumes. Significantly different means within N source treatment at $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ in bold type and separated by different letters. UV-B_{22} = 22% of ambient ultraviolet-B radiation, UV-$A_{cont.}$ = ultraviolet-A control, $PAR_{cont.}$ = photosynthetically active radiation control, DM = dry matter.

UV-B treatment	N source	Root flavanoids (Abs g^{-1})	Root anthocyanins (Abs g^{-1} DM)	Root soluble sugars (mg g^{-1} DM)	Root starch (mg g^{-1} DM)	leaf flavanoids (Abs g^{-1} DM)	Leaf anthocyanins (Abs g^{-1} DM)
				Virgilia oroboides			
$PAR_{cont.}$	Symbiotic N	63.82	0.98	12.70	97.31	114.93	0.83
	NO_3	58.80	0.98	14.90	102.19	109.25	0.86
UV-$A_{cont.}$	Symbiotic N	45.94	0.90	12.25	91.43	104.96	0.93
	NO_3	59.91	0.89	14.71	109.40	104.73	0.77
UV-B_{22}	Symbiotic N	47.20	0.94	11.87	101.40	99.13	0.68
	NO_3	53.98	0.80	18.20	98.35	103.62	0.75
Wald χ^2 statistic:							
UV-B (d.f.=2)		4.44	3.74	0.14	0.06	5.43	0.83
N (d.f.=1)		**7.69****	1.20	**7.54****	2.43	0.02	0.05
UV-BxN (d.f.=2)		3.65	1.79	1.28	4.83	0.87	2.90
				Cyclopia genistoides			
$PAR_{cont.}$	Symbiotic N	**260.72a**	35.79	26.86	92.41	485.85	0.45
	NO_3	**171.22b**	32.72	31.87	94.04	467.23	0.61
UV-$A_{cont.}$	Symbiotic N	196.08a	34.86	33.39	96.21	490.33	0.36
	NO_3	182.40a	31.85	19.48	94.24	515.83	0.26
UV-B_{22}	Symbiotic N	210.70a	39.24	19.02	95.56	474.83	0.39
	NO_3	202.18a	33.02	26.40	92.67	474.83	0.45
Wald χ^2 statistic:							
UV-B (d.f.=2)		2.43	2.07	1.83	0.39	2.17	1.81
N (d.f.=1)		**7.84***	**4.33***	0.02	2.48	0.18	0.18
UV-BxN (d.f.=2)		**6.93***	0.43	4.04	4.33	2.31	3.46
				Podalyria calyptrata			
$PAR_{cont.}$	Symbiotic N	58.56	0.54	14.73	89.64	121.17	0.00
	NO_3	48.24	0.46	11.44	81.52	117.78	0.00
UV-$A_{cont.}$	Symbiotic N	61.29	0.52	15.84	79.12	115.74	0.00
	NO_3	46.68	0.51	10.75	71.03	112.87	0.00
UV-B_{22}	Symbiotic N	59.07	0.68	15.88	86.16	138.63	0.00
	NO_3	53.82	0.44	11.79	66.93	139.65	0.00
Wald χ^2 statistic:							
UV-B (d.f.=2)		0.08	1.46	0.19	2.43	**31.60*****	-
N (d.f.=1)		2.16	**5.37***	**8.84***	3.65	1.10	-
UV-BxN (d.f.=2)		1.25	1.27	0.34	0.04	1.10	-

Appendix 19. Publications

1. **Chimphango SBM**, Musil CF and Dakora FD (2004) Response to ultraviolet-B radiation by purely symbiotic and NO_3-fed nodulated tree and shrub legumes indigenous to Southern Africa. *Tree Physiology* **24**, 181-192.

2. **Chimphango SBM**, Musil CF and Dakora FD (2003) Effect of ultraviolet-B radiation on plant growth, symbiotic function and concentration of metabolites in three tropical legumes. *Functional Plant Biology* **30**, 309-318

3. **Chimphango SBM**, Musil CF and Dakora FD (2003) Response of purely symbiotic and NO_3-fed nodulated plants of lupinus luteus and vicia atropurpurea to ultraviolet-B radiation. *Journal of Experimental Botany* **54**, 1771-1784.

4. Musil CF, **Chimphango SBM** and Dakora FD (2002) Effects of elevated Ultraviolet-B radiation on Native and cultivated plants of Southern Africa. *Annals of Botany* **90**, 127-137.

5. Musil CF, Kgope BS, **Chimphango SBM** and Dakora FD (2003) Nitrate additions enhance the photosynthetic sensitivity of a nodulated South African Mediterranean-climate legume (*Podalyria calyptrata*) to elevated UV-B. Environmental and Experimental Botany **50**, 197-210.